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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **Annual General Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University College, Gower Street, London, W.C.* 1, *on Friday,* 16 *March* 1951 *when the following papers were read:*

COMMUNICATIONS

The Enzymic Hydrolysis of Diethyl *p*-nitrophenyl Phosphate (E 600). By W. N. ALDRIDGE. (*Medical Research Council Unit for Research in Toxicology, Carshalton, Surrey*)

During work on diethyl *p*-nitrophenyl phosphate (E 600), a potent inhibitor of cholinesterase (cf. Aldridge, 1950), it was found that on the addition of E 600 to rabbit serum there was the rapid liberation of *p*-nitrophenol as shown by the appearance of a yellow colour. The activity of rabbit serum is such that 1 ml. of serum will hydrolyse under optimum conditions about 20 mg. of E 600 in 30 min. Two methods of determination of enzyme activity were used: (*a*) a method using the Warburg apparatus based on CO_2 liberation from bicarbonate by the 2 equivalents of acid liberated on hydrolysis of E 600, (*b*) a colorimetric method based on the yellow colour of *p*-nitrophenol at pH values greater than 7.

The ability to hydrolyse E 600 has been found to be shown by many tissues. Using rabbit serum as a source of the enzyme, it has been shown that the enzyme is heat-labile, has a pH optimum of 7·3–7·6, and that it is inhibited by mercury, copper and nickel (10^{-6} M), by iodoacetate (10^{-2} M) and by NaF (10^{-1} M). The enzyme is not inhibited by cyanide, cysteine, and is only slightly inhibited by 4×10^{-3} M-eserine.

Huggins & Lapides (1947) state that rabbit serum hydrolyses *p*-nitrophenyl acetate and propionate extremely rapidly. Examination of this enzyme showed that this esterase was not inhibited by 10^{-3} M-E 600. The esterase in rat serum, which also rapidly hydrolyses *p*-nitrophenyl acetate and propionate, is, on the other hand, inhibited by 10^{-8} M-E 600. Rabbit serum hydrolyses E 600 rapidly, while rat serum hydrolyses it only slowly.

Summation experiments with *p*-nitrophenyl acetate and E 600 on rabbit serum lead to the belief that the same enzyme is hydrolysing both substrates. Inhibition by E 600 has provided a tool for separating esterases into two distinct types.

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The Influence of 2:4-Dinitrophenol on the Pasteur Effect in Bull Spermatozoa. By D. R. MELROSE (*Reading Cattle Breeding Centre, Ministry of Agriculture and Fisheries*) and C. TERNER (*National Institute for Research in Dairying, University of Reading*)

Ejaculated bull semen was either diluted with phosphate saline without Ca^{++} (Krebs & Eggleston, 1940) or centrifuged as described by Tosic & Walton (1950), and the spermatozoa resuspended in phosphate saline. Samples containing 5×10^8 to 12×10^8 spermatozoa in 4 ml. were incubated in the Warburg apparatus at 37° under aerobic and anaerobic conditions. Fructose, pyruvate and lactate were estimated chemically.

Most samples showed a large Pasteur effect, the rate of fructose utilization in air being about one-half of the rate of its disappearance under strictly anaerobic conditions, but in some samples the Pasteur effect was small or absent. Lactic acid accumulated under aerobic conditions when either fructose or pyruvate was metabolized, and when both substrates were present the amount of lactic acid was increased. In the presence of fructose the addition of pyruvate had no effect on Z_{fructose} and did not increase Z_{O_2} ($Z = \mu$l./10^8 spermatozoa/hr.). The addition of 10^{-4} M-2:4-dinitrophenol (DNP) usually increased Z_{O_2} by 50–100 % when fructose or pyruvate was present; in the presence of both fructose and pyruvate the increase of Z_{O_2} was smaller or absent. Aerobic disappearance of fructose and accumulation of lactate were accelerated by DNP, and when

Table 1. *Effect of 2:4-dinitrophenol on metabolism of bull spermatozoa*

(Sample H.B.C., whole semen diluted with phosphate saline. Sample W.R. divided into two portions: (*a*) whole semen; (*b*) washed spermatozoa resuspended in phosphate saline. Aerobic incubation: gas, air; NaOH in centre well. Anaerobic incubation: bicarbonate added (final concentration 0·025 M); gas, 5% CO_2 + 95% N_2, yellow phosphorus in centre well. Incubation time: 100 min. Fumarate (0·002 M) in all aerobic cups. Substrate concentration at zero time: pyruvate (0·005 M), fructose (0·005 M). DNP (10^{-4} M). Sperm count/cup: H.B.C., $7{\cdot}26 \times 10^8$; W.R. (*a*), $7{\cdot}24 \times 10^8$; W.R. (*b*), $7{\cdot}04 \times 10^8$.)

Name of bull	Cup no.	Substrates and inhibitors	Experimental condition	(μl./10^8 spermatozoa/hr.) Z_{O_2}	$Z_{fructose}$	$Z_{pyruvate}$	$Z_{lactic\ acid}$
H.B.C.	1	Fructose	Aerobic	−15·0	−20·8	—	+29·8
	2	Fructose, pyruvate	Aerobic	−18·8	−20·6	−16·6	+36·5
	3	Fructose, pyruvate, DNP	Aerobic	−14·5	−32·0	−12·7	+52·4
	4	Fructose	Anaerobic	—	−24·4	—	+51·0
W.R. (*a*)	1	Fructose	Aerobic	−12·1	−17·2	—	+15·2
	2	Fructose, DNP	Aerobic	−28·1	−24·6	—	+33·2
	3	Fructose, nicotinamide, cozymase, DNP	Aerobic	−33·7	−25·7	—	+42·5
	4	Fructose	Anaerobic	—	−42·2	—	+85·4
W.R. (*b*)	5	None	Aerobic	−6·7	—	—	—
	6	Pyruvate	Aerobic	−10·8	—	−35·2	+21·2
	7	Pyruvate, DNP	Aerobic	−18·7	—	−12·4	+4·3

pyruvate was also present, the anaerobic rates of fructolysis and lactic acid formation were sometimes exceeded. The rate of fructolysis greatly exceeds the rate of pyruvate oxidation; this accounts for the accumulation of lactic acid in semen under aerobic conditions (Table 1). In the presence of 0·01 M-fluoride, aerobic lactic acid formation was strongly inhibited, but was partly restored on addition of pyruvate and further increased by DNP.

Stimulation of respiration and increased aerobic lactic acid formation under the influence of DNP has been reported by Lardy, Hansen & Phillips (1945). They concluded 'that the preliminary dehydrogenations as well as the subsequent decarboxylative dehydrogenations are accelerated by DNP'. The mechanism of the action of DNP on glycolytic and oxidative reactions and on the Pasteur effect in spermatozoa was discussed.

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The Point of Action of 2:4-Dinitrophenol in the Inhibition of Oxidative Phosphorylation. By C. Terner. (*National Institute for Research in Dairying, University of Reading*)

It was previously reported (Terner, 1950) that 2×10^{-4} M-2:4-dinitrophenol (DNP) increases the Q_{O_2} (μl. O_2/mg. dry wt./hr.) of lactating mammary-gland slices metabolizing pyruvate without correspondingly increasing the rate of pyruvate utilization, thus causing more complete oxidation of pyruvate. In the presence of glucose or lactate, however, 2×10^{-4} M-DNP did not increase Q_{O_2} and often caused definite inhibition. In order to test whether cozymase is involved, tissue slices, after preliminary incubation for 60 min. at 37° without added substrates, were incubated with pyruvate or glucose, and the effect of low concentrations of DNP and of added cozymase was studied.

5×10^{-5} M-DNP usually increased Q_{O_2} of mammary tissue by about 100% in glucose or pyruvate, while 2×10^{-4} M-DNP increased Q_{O_2} in pyruvate but inhibited in glucose. In some experiments in which 5×10^{-5} M-DNP inhibited Q_{O_2} in glucose, the addition of $1{\cdot}25 \times 10^{-4}$ to $2{\cdot}5 \times 10^{-4}$ M-cozymase reversed the inhibition. When glucose and pyruvate were metabolized at the same time by mammary-gland slices or brain cortex slices, the addition of cozymase caused increased accumulation of lactic acid which was greatly accelerated by the further addition of 2×10^{-4} M-DNP (Table 1).

The results indicate that when pyruvate and cozymase are available, the anaerobic oxidation-reduction step of glycolysis can operate also under aerobic conditions. Friedkin & Lehninger (1949) and Lehninger (1949) have demonstrated the esterification of inorganic phosphate during electron transport between reduced cozymase and oxygen and found DNP to be a powerful inhibitor of coupled phosphory-

Table 1. *Effect of cozymase and 2:4-dinitrophenol on metabolism of lactating mammary tissue*

(Rabbit mammary-gland slices, incubated at 37° in phosphate saline without Ca^{++} (Krebs & Eggleston, 1940): gas, 100% O_2; fumarate (0·002M) in all cups. Additions: pyruvate (0·005M), glucose (0·005M), nicotinamide (0·01M), cozymase ($2·5 \times 10^{-4}$M), DNP (2×10^{-4}M). $Q_{\text{lactic acid}}$ corrected for lactic acid found in absence of added substrates.)

Cup no.	Additions	Q_{O_2}	Q_{pyruvate}	Q_{glucose}	$Q_{\text{lactic acid}}$
1	Glucose	−5·2	—	−2·0	+0·3
2	Glucose, nicotinamide, cozymase	−6·2	—	−1·7	+0·4
3	Glucose, nicotinamide, cozymase, DNP	−3·2	—	−0·1	+0·3
4	Glucose, pyruvate	−6·3	−5·8	−1·5	+1·0
5	Glucose, pyruvate, nicotinamide, cozymase	−6·7	−6·1	−1·3	+1·6
6	Glucose, pyruvate, nicotinamide, DNP	−8·8	−4·7	−0·8	+1·4
7	Glucose, pyruvate, nicotinamide, cozymase, DNP	−10·1	−5·7	−1·4	+3·3

lation in their enzyme system. The present results suggest that the primary effect of DNP may be the inhibition of the aerobic oxidation of reduced cozymase. The inhibition by DNP of the Pasteur effect and of oxidative phosphorylation may thus be due to a common mechanism. Since it was found that 2×10^{-4}M-DNP has no effect on the oxidation of added succinate by mammary tissue, the point of action of DNP would lie between cozymase and the cytochrome system. DNP may also be expected to inhibit the aerobic oxidation of pyridine nucleotides catalysing reactions of the Krebs cycle. It is suggested that in the presence of DNP, electron transfer from the pyridine nucleotides to oxygen may be partly replaced by a series of oxidation-reduction reactions, while steps of the Krebs cycle not requiring pyridine nucleotides can transfer electrons to oxygen. Anaerobic oxidation-reduction reactions resulting in the formation of succinate from oxaloacetate have been described by Hunter (1949).

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A Comparison between Glucose and Acetate as Precursors of Milk Fat in the Rabbit. By T. H. French* and G. Popják. (*The National Institute for Medical Research, Mill Hill, N.W.7*)

Rabbits during the third week of lactation were given by stomach tube [1-^{14}C]-acetate followed by ^{14}C-starch 3–5 days later, or were injected intravenously with [1-^{14}C]-acetate followed by ^{14}C-glucose 3 days later when the radioactivity in the milk fat due to acetate had fallen to a negligibly low level. At the time of administration of the labelled substance the mothers were separated from their litters; they were milked 6 hr. later. Usually 20 ml. of milk were obtained. The milk fat was extracted by boiling with ethanol-ether mixture 3:1 (v/v) and the fatty acids fractionated as previously described (Popják, French & Folley, 1950, 1951). In addition to the fatty acids, glycerol was obtained as the tribenzoate from the hydrolysate of glyceride fat and also lactose from the ethanol-ether extract. Cholesterol was isolated as the digitonide.

Table 1 shows that acetate is used for the synthesis of milk fatty acids just as in the ruminant (cf. Popják *et al.* 1950, 1951). Moreover, the fatty acid fractions containing the shorter-chain acids (water-soluble and insoluble steam-volatile acids) had the highest specific activity closely resembling the results obtained on the goat. As far as the fatty acids and cholesterol are concerned the similarity between the results obtained after the administration of carbohydrate and acetate is striking. It is inferred that glucose also must yield C_2 units, which are indistinguishable from those derived from acetate. Bloch & Rittenberg (1944) have shown that only those compounds which give rise to acetate (or C_2 units) are used for cholesterol synthesis. However, carbohydrate appears to be a better source of 'fat', since it also acts as a direct precursor of glycerol. If the specific activity of the lactose can be taken as the specific activity of the hexose available in the mammary gland as substrate, then the specific activity found for glycerol indicates that 50–100% of the glycerol in the milk is newly formed in the mammary gland. It does not seem unlikely that the availability of glycerol is one of the limiting factors in fatty acid synthesis, and that the enhancing effect of carbohydrate on the *in vitro* synthesis of fatty acids from acetate described by Bloch & Kramer (1948) and by Folley & French (1949) may be due to glycerol formation.

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Table 1. *Specific activities of milk constituents (counts/min./mg. C in infinite thickness) from rabbits after administration of* [1–^{14}C]*-acetate or* [^{14}C]*-carbohydrate*

Rabbit no.	Labelled substance administered	Dose of ^{14}C (μc.)	Lactose	Glycerol	Steam volatile fatty acids: Water sol.	Steam volatile fatty acids: Water insol.	Non-volatile fatty acids	Cholesterol
16	Starch (12 mg.)	12	1,385	965	364	302	56	—
17	Acetate (4·1 mg.)	50	539	486	2880	2760	355	234
	Starch (37·3 mg.)	50	11,760	13,332	2855	2740	276	372
18	Acetate (4·1 mg.)	50	161	—	2068	2158	432	169
	Glucose* (357 mg.)	50	5,560	3883	1430	1520	324	299

* Milk collected during 12 hr., all others 6 hr.

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The Rapid Enzymic Breakdown of a Constituent of Kephalin by Brain Homogenates. By G. H. Sloane Stanley. (*Institute of Psychiatry, Maudsley Hospital, London, S.E.* 5)

Until recently, the reported rates of phospholipin metabolism in adult brain were low, both *in vivo* and *in vitro* (e.g. Changus, Chaikoff & Ruben, 1938; King, 1931; Sperry, Brand & Copenhaver, 1942; Tyrrell, 1950); the *in vitro* results correspond to a rate of about 0·0003–0·0016 μmol./mg. dry wt./hr.

However, Sperry (1947) reported a faster loss of phospholipin from brain suspensions in bicarbonate buffer (about 0·01 μmol./mg. dry wt./hr.); and Dawson & Richter (1950) have found a rate of phospholipin phosphorus turnover in the brain *in vivo* of about 0·005 μmol./mg. dry wt./hr.

In the experiments to be described, suspensions of various fractions of kephalin prepared according to Folch (1942, 1948), and of other brain lipids obtained as by-products, were added to homogenates of fresh adult guinea pig brain in Warburg flasks, and the resultant gas changes followed for from 3 to 4 hr.

The brain homogenates and the lipid suspensions were prepared in 'Krebs salt solution' (Dixon, 1943) with KH_2PO_4 omitted; the gas in the flasks was N_2(95 %)+CO_2(5 %), and the bath temperature was 37°. All samples of fraction I of kephalin caused a rapid evolution of CO_2 in addition to that due to the homogenates alone; other lipids gave small or negligible increases. The reaction was almost completely abolished by preheating the brain homogenates to 100° for 5 min.; its rate became proportional to the tissue concentration when this was below about 20 mg./ml., and was then 0·04–0·19 μmol. CO_2/mg. fresh tissue/hr. (roughly equivalent to 0·20–0·95 μmol./mg. dry wt./hr.).

Preliminary attempts to analyse this effect have given evidence for the formation of acid-soluble phosphate and inositol during the reaction; there was no evidence for the release of any nitrogenous compounds.

These results suggest that the substance attacked by the brain homogenates was diphosphoinositide, the chief single constituent of fraction I of kephalin (Folch, 1949).

It is felt, however, that the chief interest of these results lies in the high rate of the reaction; it is similar to that of other rapid enzymic processes known to occur in brain, such as respiration and the breakdown of cozymase (McIlwain & Rodnight, 1949). The other essential difference between these experiments and those quoted above, with the exception of King's (1931) work, is that substrates added to the brain homogenates from without were used, besides the intrinsic phospholipids of the tissue.

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Antitryptophanase. By Doris E. Dolby and F. C. Happold. (*Department of Biochemistry, School of Medicine, Leeds*)

Kümmerling (1943) prepared killed suspensions of *Escherichia coli*, Proteus X 19, and certain vibrios which possessed tryptophanase action. The techniques employed were those originally used by Happold & Hoyle (1934, 1935). He showed that the tryptophanase from *Esch. coli* could be inhibited by an anticoli serum, and that the Proteus X 19 tryptophanase could be similarly inhibited by the serum of typhus patients which gives a positive Weil Felix reaction. He also suggested that the antitryptophanase of each species shows a separate species specificity, but proof for this claim is incomplete.

The present authors, in complete ignorance of the work of Kümmerling, prepared an antitryptophanase (and certain other anti-enzymes) by injecting tryptophanase into rabbits. They have used the KCl extraction technique on acetone dried cells (Dawes, Dawson & Happold, 1947). The extracts were dialysed in the cold to remove K^+ and the resultant crude trytophanase used as antigen.

Protein fractions extracted in similar manner from *Esch. coli* cells grown on media which contain (*a*) glucose, phenylalanine and/or tyrosine as well as tryptophan, and (*b*) gelatin hydrolysates, have no tryptophanase activity, but do contain other enzymes (Evans, Handley & Happold, 1941; Dawes & Happold, 1949). Antisera prepared against such proteins naturally precipitate with the homologous proteins and cross-precipitate with each other. Cell-free tryptophanase is inhibited by antitryptophanase; it is not reduced in activity by the other two types of antisera though protein precipitation occurs. Similarly, when the antitryptophanase serum is tested with the proteins extracted from the non-tryptophanase-containing cells, precipitation occurs; this antibody absorption does not remove the antitryptophanase activity of the sera, though it may remove other anti-enzymes common to all three types of antiserum.

Tryptophanase can be redissociated from the inactive tryptophanase-antitryptophanase complex with 0·1 N-NH_4OH. The complex appears stable and remains inactive under the conditions of the experiment at pH 7·0.

The 'X' antigens of the Salmonella group (Topley & Ayrton, 1924; Happold, 1928, 1929), whose production by the living organism is conditioned by controlled changes in the cultural conditions, might be adaptive enzymes. The above immunological approach might add to our knowledge of adaptive enzyme structure, and this general question is being examined further.

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Studies on Rat-liver Phosphatase. By G. A. J. Goodlad, G. T. Mills and Evelyn E. B. Smith. (*Biochemistry Department, University of Glasgow*)

The aim of the present work is an attempt to throw more light on the metabolic role of liver phosphatases. The phosphatases studied were those having pH optima for the hydrolysis of sodium β-glycerophosphate at 3·75, 5·5 and 10·5.

The activities of the three phosphatases have been determined in liver tissue during the growth of the rat from birth to maturity, and the results expressed both in relation to wet tissue weight and to the tissue deoxyribonucleic acid (DNA). Davidson & Leslie (1950*a*, *b*) consider that in view of the suggested constancy of the DNA content of the cell nucleus of a single species, the DNA content of a sample of tissue can be used as a measure of the number of cells present in the sample. It follows that when the activity of an enzyme in a tissue homogenate is related to the DNA content of the tissue, an indication is obtained of the enzyme activity per cell.

This suggestion has been utilized in the present work, and it is deduced from our results that the two acid phosphatases accumulate in the cell at a greater rate than the alkaline phosphatase, and also that the rate of accumulation of alkaline phosphatase is the same as that of ribonucleic acid (RNA).

During the period of rapid growth of the liver following subtotal hepatectomy (2–4 days) there is a rapid increase in alkaline phosphatase activity to a maximum value, at between 2 and 3 days, of 350% of the original activity. This is followed by a fall and then a rise to a subsidiary peak of 100% above normal activity at about 7 days after the operation. The level of alkaline phosphatase activity reaches normal values after 10–12 days. The peaks of alkaline phosphatase activity correspond to the peaks of RNA concentration in the liver during regeneration. The acid phosphatase activities show no significant change from normal. These results are in confirmation of those of Oppenheimer & Flock (1947) and Norberg (1949).

During the development of the fatty liver produced on a low protein, low choline diet, the alkaline and pH 3·75 acid phosphatases show no significant alteration in activity from normal. The pH 5·5 phosphatase, however, showed an increase in activity of 50% over normal by the 20th day on the diet, when the livers contained considerable amounts of fat.

A possible conclusion from this preliminary work is that alkaline phosphatase is connected with RNA synthesis or turnover (cf. Davidson, 1949).

The authors wish to thank Dr W. C. Hutchison for some of the DNA and RNA values reported in this communication.

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The Electrophoresis of Enzymes on Filter Paper. By G. T. Mills and Evelyn E. B. Smith. (*Biochemistry Department, University of Glasgow*)

The successful electrophoresis of proteins on filter paper has recently been reported by Cremer & Tiselius (1950), Durrum (1950) and Turba & Enenkel (1950), and the present communication is an extension of this work to the field of enzymology.

The apparatus used is a slight modification of that of Cremer & Tiselius (1950), in which no special cooling arrangement is used. The filter paper used is Whatman 3 MM in strips 3 or 4 cm. wide, the other conditions being similar to those employed by Cremer & Tiselius (1950).

Enzymic activity is localized by means of the chromogenic substrates, phenolphthalein glucuronide for glucuronidase, phenolphthalein phosphate for phosphatase (cf. Talalay & Huggins, 1945), and *p*-nitrophenyl butyrate for esterase (cf. Huggins & Lapides, 1947). A strip of filter paper soaked in substrate and buffer is placed in contact with the electrophoretic strip, the two clamped between glass plates, incubated, and the colour developed after a suitable time. Protein is localized on replicate strips by the methods of Durrum (1950) or Turba & Enenkel (1950).

A diagram resembling the conventional electrophoretic schlieren diagram may be obtained by sectioning the paper strip into 5 mm. lengths and assaying each for enzymic activity, after which the activity is plotted against distance migrated. By this technique the multiple nature of an enzyme may be demonstrated as has been done for ox-spleen β-glucuronidase.

In order to estimate the non-electrophoretic movement of protein, due to electro-osmosis, the movement of a spot of glucose is determined by the usual chromatographic techniques.

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Choline Metabolism in the Embryo Chick. By G. S. Boyd. (*Department of Biochemistry, University of Edinburgh*)

Previous experiments (Boyd, 1950) have shown that during the development of the chick embryo there is an overall decrease of about 25% of the 'labile methyl groups' in the egg, accounted for almost entirely by the disappearance of choline. The methionine content varies little, and the accumulation of creatine, creatinine and other known methylated compounds accounts for less than 10% of the choline metabolized, even on the basis (Du Vigneaud, Chandler, Simmonds, Moyer & Cohn, 1946) of one 'labile methyl group' per choline molecule.

It has now been shown that the liver of the chick embryo contains the choline oxidase enzyme system (cf. Williams, Sunde, Cravens & Elvehjem, 1950), and that the liver can methylate homocysteine to yield methionine. It is concluded that the liver contains a transmethylase, and that transmethylation in the chick embryo follows the same course as in the rat (Dubnoff, 1949; Muntz, 1950). Since dimethylaminoacetic acid does not accumulate in the system in quantities detectable by paper partition chromatography, the demethylation product must be rapidly metabolized, perhaps by oxidative demethylation to formaldehyde and glycine, as suggested by Handler, Bernheim & Klein (1941).

There remains a substantial 'loss' of choline which must from its very magnitude represent an important metabolic process. To date, no evidence bearing directly on this point is available, but a possible alternative metabolic pathway is suggested by recent isotope work, such as that of Siekevitz & Greenberg (1949, 1950). These workers have shown that in rat-liver slices the methyl carbon of choline appears as the β-carbon of serine.

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Resistance of Acetylcholine Esterase in Dry Preparations to Certain Organic Solvents. By K. Bullock. (*Department of Pharmacy, University of Manchester*)

Following attempts to dehydrate and defat brain tissue in the usual ways, Bernheim & Bernheim (1936) reported that ethanol, acetone and ether were poisonous to brain cholinesterase. Nachmansohn & Lederer (1939) found that acetone, ethanol and methanol completely inactivated the acetylcholine esterase (AChE) of fresh nerve tissue. In view of the increased resistance to heat of enzyme systems (Bullock, 1947) and bacteria (Bullock & Lightbown, 1947) when in the dry condition, it was decided to determine the effects of light petroleum (b.p. 40–60°), diethyl ether, acetone and ethanol on the AChE in powders produced by drying *in vacuo* over P_2O_5 thin layers of rabbit brain ground to a cream with 4% of neutral acid-extracted sand. Solvents were purified and dehydrated before use. Portions (0·5 g.) of the brain powder were extracted by stirring and centrifuging three times with the solvent, the total time of exposure to the solvent being approximately 30 min. The extracted powder was again dried over P_2O_5, and its AChE activity compared with that of the unextracted powder using Warburg manometers at 37°. The Warburg flasks contained 6·25 mg. of dried brain in a total volume of 3 ml. of liquid under 5% CO_2 in N_2. Final concentrations were: $NaHCO_3$, 0·025 M; NaCl, 0·15 M; acetylcholine, 0·015 M; $MgCl_2$, 0·04 M.

Table 1. *Acetylcholine esterase* (AChE) *activity of dry rabbit-brain tissue and the effects of extraction with fat solvents* (AChE *activities are expressed in* μ*l.* CO_2 *produced by* 1 *mg. of dry powder in* 60 *min. The dry brain powder contained* 21·1% *total ash;* 15·4% *acid insoluble ash*)

		AChE activity			
Solvent	Lipid extracted (%)	Unextracted powder	Extracted powder	*Supernatant from extracted powder	Solubility AChE (%)
Light petroleum, b.p. 40–60°	19·5	48·8	63·2	31·5	49·9
Diethyl ether	19·5	49·3	66·8	37·9	56·7
Acetone	8·0	51·7	56·0	25·3	45·2
†Ethanol	25·2	46·82	55·0	18·1	32·9
			Supernatant from unextracted powder		
Unextracted powder (mean of 4 determinations)		49·8	18·7		37·6

* μl. CO_2 produced in 60 min. by the supernatant fluid from 1 mg. of dry tissue after centrifuging a 1·25% (w/v) suspension.
† A different sample of brain powder was used with ethanol.

Extractions were carried out in duplicate, and the AChE activity of each suspension or solution was determined in duplicate. To test the solubility of the AChE, 1·25% (w/v) suspensions in the above salt solution were centrifuged at 1500 ***g*** for 5 min., and the AChE activity of the opalescent supernatant fluid compared with that of the suspension. The results are shown in Table 1. The dry powders are more active after extraction with light petroleum, diethyl ether or acetone by an amount approximately proportional to the percentage of lipid material removed. Further experiments confirmed this even after contact for 72 hr. between the dry powder and acetone or ether. The percentage solubility of the AChE is increased in the fat-free powders. The powders are relatively stable over a period of several months at 0° and form, especially the ether-extracted powder, a convenient source of AChE. With ethanol the extracted powder does not quite show the increased activity which would correspond to the percentage of lipids removed although it is more active than the original powder. After contact for 72 hr. with ethanol the powder had lost 22% of its original activity corresponding to a 42% loss on a lipid-free basis.

Two facts are obvious: (1) that drying greatly increases the stability of AChE to organic solvents, and (2) that the lipids extracted by light petroleum, ether or acetone are not essential to the functioning of AChE.

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Activation of the Heart-muscle Succinic Oxidase System. By W. D. Bonner, Jun. (U.S. Public Health Service Fellow). (*Molteno Institute, University of Cambridge*)

Keilin & Hartree (1949) have shown that succinic oxidase preparations which have been inactivated by various means can be reactivated by certain inert substances, e.g. denatured globin and tricalcium phosphate gel. Bonner (1950) found that preparations which contained little inorganic phosphate (4 μg. P/mg. dry wt.) and which showed very low activity without added phosphate can be activated completely by denatured globin. In further experiments with a variety of proteins, only denatured globin was found to be an effective activating agent. Tricalcium phosphate gel was one-half to one-third as effective. A distinguishing feature of denatured globin is its high content of histidine and lysine, a feature shared by few other proteins. Therefore these amino-acids were tested and were found to be very effective in activating the low-phosphate preparation. Of a large number of amino-acids tested only histidine and lysine were as effective as denatured globin, aspartic acid was approximately one-half as effective and the remainder were inert in this regard. Various substituted imidazoles, glyoxalines, and urea

derivatives were also tested, and of these only glyoxaline-4:5-dicarboxylic acid showed an activity equivalent to that of denatured globin.

The low-phosphate preparation, activated by histidine, lysine, or denatured globin, has been compared manometrically and spectroscopically with the normal preparation in phosphate. The two preparations behave identically in every respect, except that the low-phosphate preparation is not inhibited by fluoride. The dependence of fluoride inhibition on the presence of phosphate will be the subject of a future communication.

The observation of Keilin & Hartree (1949) that succinate was oxidized in the almost complete absence of inorganic phosphate is particularly interesting, since it is now established that, in mitochondrial preparations, the oxidation of succinate to fumarate is coupled with the esterification of inorganic phosphate (Cross, Taggart, Covo & Green, 1949). The possibility that the considerable amounts of combined phosphorus found by Ball & Cooper (1949) might provide catalytic amounts of inorganic phosphate for the oxidation of succinate has been tested. By extracting washed heart muscle with borate buffer and repeated sedimentation by acidification and high-speed centrifugation, a preparation containing only 0·05 μg. inorganic P/mg. fat-free dry weight was obtained. At the dilution employed in the manometric test, the total concentration of inorganic phosphorus provided by the preparation and other reagents was only $0{\cdot}93 \times 10^{-6}$M. The Q_{O_2} (μl. O_2/hr./mg. fat-free dry weight) was 457 at 38°, in the presence of borate buffer, pH 7·3, and histidine. With added inorganic phosphate (0·13M) the Q_{O_2} was 411. Incubation of the concentrated preparation with succinate at 38° for 30 min. liberated negligible amounts of inorganic phosphorus. These findings confirm the conclusion of Keilin & Hartree (1949) that inorganic phosphorus is not necessary for the oxidation of succinate. Dihydrocozymase was also oxidized under the same conditions as rapidly in the absence as in the presence of added phosphate.

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Excretion of Conjugated Phenylacetic Acid in Phenylketonuria. By L. I. Woolf. (*Institute of Child Health, The Hospital for Sick Children, Great Ormond Street, London, W.C.*1)

In phenylketonuria, phenylalanine is not converted to tyrosine, but is instead excreted partly unchanged and partly converted to phenylpyruvic and phenyllactic acids (Fölling, 1934; Jervis, Block, Bolling & Kanze, 1940). It has been suggested (Neuberger, 1950) that phenylpyruvic acid should be partially converted *in vivo* to phenylacetic acid, which in humans would be excreted conjugated with glutamine (Thierfelder & Sherwin, 1914; cf. Lewis & Chandler, 1932). In the present investigations phenylacetylglutamine has been found in these urines in considerable amounts.

Paper chromatograms of urine from these patients show, on ninhydrin development, a large spot due to phenylalanine and a faint blue spot near it. A spot in the same position appears in paper chromatograms of the urine of a normal subject fed phenylacetic acid.

Phenylacetylglutamine is only with difficulty extracted by ether from acidified aqueous solution (partition coefficient 0·01 at pH 1), but is more readily extracted by ethyl acetate (partition coefficient 0·17 at pH 1). The urines were therefore brought to pH 1 and extracted with ether to remove phenylpyruvic acid and phenyllactic acid, the phenylacetylglutamine was then extracted with ethyl acetate and estimated by the Kapeller-Adler reaction (Block & Bolling, 1939). A correction is made for the amount extracted by the ether. Phenylacetylglutamine gives, mol. for mol., 0·89 of

Table 1. *Urinary excretion of phenylacetylglutamine and other compounds by phenylketonurics*

Patient	Body weight (kg.)	24 hr. urine vol. (ml.)	(mg./100ml.) Phenyllactic acid	Phenylacetyl-glutamine	Phenylalanine	Phenylpyruvic acid
1	27·5	1170	66	25	24·6	50
2	16	570	46	58	22·5	60
Mean of 8 patients	—	—	54	53	38	50

the colour given by phenylalanine. A semi-quantitative alternative is to estimate on a paper chromatogram the glutamic acid released on acid hydrolysis of the ethyl acetate extract.

Jervis *et al.* (1940) determined phenylalanine excretion by applying the Kapeller-Adler reaction to the residue after ether extraction of such urines; their results for phenylalanine must be high owing to the low solubility of phenylacetylglutamine in ether.

Phenylacetic acid is toxic, affecting the central nervous system (Sherwin & Kennard, 1919). The presence of this acid in the system may explain the relation between the metabolic error and the associated mental defect.

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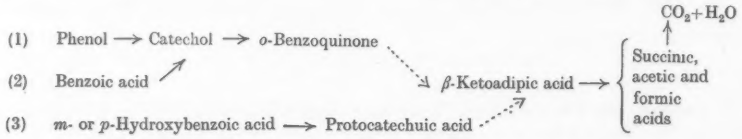
The Oxidation of Aromatic Compounds by Soil Bacteria. By W. C. Evans and B. S. W. Smith.
(*Agricultural Biochemistry Section, University College of Wales, Aberystwyth*)

The metabolic pathway through which certain soil bacteria oxidize aromatic compounds has been partially elucidated in the case of the simpler substances, e.g. phenol, benzoic acid and its hydroxylated derivatives, as follows:


(1) Phenol → Catechol → o-Benzoquinone
(2) Benzoic acid
(3) m- or p-Hydroxybenzoic acid → Protocatechuic acid
β-Ketoadipic acid
CO2+H2O
Succinic, acetic and formic acids

Proof of the formation of these intermediates rests on their isolation from cultures starting with the initial substrates as sole source of organic carbon in simple mineral media. Evans & Happold, 1939; Evans, 1947; Kilby, 1948; Evans, Parr & Evans, 1948; Parr, Evans & Evans, 1949). Their participation in the reaction chain is deduced, since they obey the criteria of simultaneous adaptation (Stanier, 1947, 1948, 1950). Hitherto, the steps from the '*o*-dihydroxybenzene' stage leading to β-ketoadipic acid, and involving the fission of the aromatic ring, remained unknown. Further hydroxylation of the aromatic nucleus became an unlikely possibility, since neither hydroxyquinol, 1:2:3:4-tetrahydroxybenzene, or 2:5-dihydroxy-1:4-benzoquinone in (1) and (2), nor any of the three isomeric trihydroxybenzoic acids in (3), conformed to the above criteria. There was also the negative evidence that no trace of any additional polyphenolic substances could be detected when active cell-free enzyme preparations from the bacteria acted on catechol to give β-ketoadipic acid, as shown by the sensitive method of chromatographic separation of phenols developed by Evans, Parr & Evans (1949).

We have now found that all the typical soil aromatic ring-splitting organisms (*Vibrio* 01, *Pseudomonas fluorescens* A 3/8 and A 3/9, etc.) will grow in a simple mineral medium with *cis-cis*-muconic acid as the sole source of organic carbon, but not with the *cis-trans* or the *trans-trans* geometric isomers of this acid (for the chemical properties of these acids see Elvidge, Linstead, Sims & Orkin, 1950). Furthermore, *cis-cis*-muconic acid cultures of these organisms give a definite Rothera reaction at a particular stage during growth. Table 1 summarizes results obtained with suspensions of

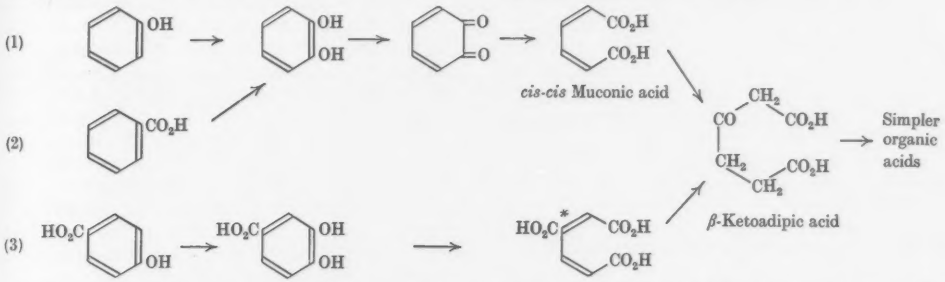
Table 1. *Action of freeze-dried bacterial cells resuspended in* 0·05 M-*phosphate buffer, pH* 7, *on various substrates*

(*a*) Cell preparations grown on phenol, catechol or benzoate.
(*b*) Cell preparations grown on *p*-hydroxybenzoate, or protocatechuate.

Substrate	Adaptation (*a*)	Adaptation (*b*)	β-Ketoadipate (isolated) (*a*)	β-Ketoadipate (isolated) (*b*)
Phenol	+	−	+	−
Catechol	+	−	+	−
Benzoate	+	−	+	−
p-Hydroxybenzoate	−	+	−	+
Protocatechuate	−	+	−	+
cis-cis-Muconate	+	−	+	−
cis-trans-Muconate	−	−	−	−
trans-trans-Muconate	−	−	−	−

freeze-dried cells prepared from *Vibrio* 01 and *Pseudomonas fluorescens* strains A 3/8 and A3/9, when acting upon various substrates.

The sequence of intermediates produced in the bacterial oxidation of these aromatic compounds may now be depicted as follows:



From these results it is clear that (1) *cis-cis*-muconic acid is a product of ring cleavage prior to the formation of β-ketoadipic acid in the bacterial oxidation of phenol, catechol and benzoic acid, and (2) *cis-cis*-muconic acid does not appear in the metabolic pathway of *p*-hydroxybenzoic or protocatechuic acid by these bacteria.

Further work is required on pathway (3), but there seems to be little doubt that a substituted muconic acid is the first product of ring cleavage; this postulated intermediate (see formula) cannot be decarboxylated directly to *cis-cis*-muconic acid, but must undergo some further change before losing this β-carboxy group.

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A Study of some Fluorescent Compounds of Oestrone, Oestradiol and Oestriol. By HANNELORE BRAUNSBERG. (*University College Hospital Medical School, London, W.C.*1)

Methods for the fluorimetric determination of oestrone, oestradiol and oestriol have been described by Jailer (1947), Bates & Cohen (1947), Finkelstein, Hestrin & Koch (1947) and Garst, Nyc, Maron & Friedgood (1950). Fluorescent compounds of undetermined structure are obtained on reaction with sulphuric acid, phosphoric acid or phthalic anhydride, under a given set of conditions, and their concentration is determined fluorimetrically. Bates & Cohen (1950) have carried out a detailed study of their method and have determined the optimum conditions for fluorimetry. Engel, Slaunwhite, Carter & Nathanson (1950) use a modification of the method of Bates & Cohen (1947). In Table 1 are shown the sensitivities for the three oestrogens relative to oestrone obtained in these methods.

The relative sensitivities will depend on the nature of the compounds formed, and on the primary and secondary filters used in the determinations. The most remarkable difference is that between the methods used by Bates & Cohen and Engel *et al.*, which cannot be explained easily by the slightly different acid concentrations, temperatures, and primary filters used.

Table 1. *Sensitivities of oestrogens when determined fluorimetrically by various methods*

	Jailer	Bates & Cohen	Engel* *et al.*	Finkelstein *et al.*	Garst* *et al.*
Oestrone	1·0	1·0	1·0	1·0	1·0
Oestradiol	1·0	0·94	0·56	2·7	0·7
Oestriol	0·05	0·34	0·70	0·75	0·3

* Calculated approximately from the calibration curves given.

The amount of fluorescent radiation is directly proportional to the amount of fluorogenic radiant energy absorbed. A study of the absorption spectra

of the compounds formed in the Finkelstein *et al.*, Bates & Cohen and Engel *et al.* methods has revealed some interesting differences which are in keeping with the relative sensitivities observed. The effect of water, alcohol and toluene on the absorption curves has also been studied and is of some interest from a practical point of view. There is a definite relationship between these absorption spectra and those of the final compounds obtained in the reaction of Kober (1931, 1938).

The absorption spectra have proved useful in the choice of a suitable light source and of primary filters. As has been shown (Braunsberg & Osborn, 1951) the intensity of light incident on the fluorescent material has a marked effect not only on the sensitivity, but also on the range of concentrations over which the fluorimetric method is applicable if the straight-line relationship between fluorescence and concentration is to hold. This range can also be calculated readily from the absorption data. Finally, the extinction coefficients of reagents and impurities may be no less important than any blank fluorescence in determining the fluorescence of the solution. This is of interest in the application to biological extracts.

The methods of Jailer and of Garst *et al.* have not been studied. The former may be expected to produce compounds similar to those of Bates & Cohen.

Specificity tests have little meaning unless they are considered in relation to absorption and fluorescence spectra and to the filter system used.

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The Collagen Content of Mammalian Bones. By H. J. Rogers, S. M. Weidmann and A. Parkinson. (*Biological Research Unit, Dental School and Hospital, University of Leeds*)

Examination of the shaft and cancellous parts of the long bones, the scapulae and vertebrae of rabbits, together with the femora of oxen and of humans, has shown that from 90 to 97% of the nitrogen in thoroughly cleaned bones exists as a protein which can be removed from the insoluble decalcified matrix by treatment in an autoclave (2 hr. at 120°) and subsequently precipitated by tannic acid. This protein, which is assumed to be collagen, accounts for more, rather than less, of the nitrogen in bones and parts of bone with a low Ca:N ratio, and hence such structures can be said to be poorly calcified. The concentration of phenolic substances in crude autoclaved extracts from bone matrix and the amount of material left insoluble after autoclaving are lower in those bones with a relatively high nitrogen content. It is suggested that this is due to the smaller number of blood vessels present in poorly calcified bone. The walls of such vessels might be expected to contribute elastin and the contents (i.e. blood) aromatic amino-acids or polypeptides and proteins containing relatively high concentrations thereof.

The Ca:N ratio of mid-femur samples from human subjects increases with age rapidly up to 20–25 years and more slowly thereafter. The proportion of the total nitrogen in these samples which is accountable as collagen remains constant, and it can, therefore, be said that the degree of calcification, even in the middle of a long bone, increases with age.

The Polysaccharide Associated with the Organic Matrix of Bone. By H. J. Rogers. (*Biological Research Unit, Dental School and Hospital, University of Leeds*)

Reports in the early literature refer to the presence of small amounts of a mucopolysaccharide in bone (Seibert & Gies, 1904). The present work was undertaken with three aims in view: (1) to determine whether or not a polysaccharide is present in bone; (2) to obtain some information of its nature if present; (3) to estimate roughly the amount in whole bone.

The preparation of the polysaccharide was as follows. Ox shaft bone was thoroughly cleaned of all

soft tissue, dried and powdered in a hammer mill, and the powder was decalcified at 5–10° for 18 hr. with N-HCl. The resulting matrix was washed thoroughly, and the acid which it had absorbed and occluded was neutralized. It was then suspended in an equal volume of water, and a rapid stream of nitrogen passed through the mixture while it was heated to 90° in a water bath for 1 hr. The resulting mixture was then adjusted to pH 8 and treated for 18 hr. at 37° with a partially purified trypsin preparation. This preparation was substantially free of substances containing hexosamine of which there are considerable amounts in the crude material. The resulting hydrolysate was centrifuged and the supernatant dialysed against running tap water for 24 hr. The non-dialysable material was concentrated *in vacuo* to about 10% of its original volume. Nitrogen was again used as a gas stream in this process. The resulting syrup was repeatedly precipitated with alkaline ethanol at 15° in the presence of 10% potassium acetate. Each precipitation was made at that concentration of alcohol which gave optimal yield and degree of purification. A solution of the final preparation was then repeatedly shaken with amyl alcohol and chloroform until no further layer of denatured protein was formed. The polysaccharide was finally precipitated once more with alkaline ethanol.

In two successive batches of the material, the constituents determined were present in the following proportions: 0·97, 0·94 mols. hexosamine HCl; 1·02, 1·07 mols. hexuronic acid, 1·68, 1·84 atoms N; 1·11, 0·98 atoms S. The absolute hexosamine concentration of several batches varied between 28 and 32% of the ash-free dried material. The relatively high nitrogen content of these and other preparations indicates that they are unlikely to be homogeneous. Moreover, although protein-free according to biuret tests they give strongly positive ninhydrin spots on paper chromatograms after acid hydrolysis.

Previous results (Rogers, 1949) for the amount of hexosamine in the whole bone have been confirmed and indicate the presence of about 0·3–0·4% of a polysaccharide of the above type in ox shaft bone. It has, however, never been possible to isolate more than about 10% of this amount using the above method. Investigations are at present being made to show how far the remaining hexosamine is part of the same or another polysaccharide.

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The Effect of Sex Hormones on the Distribution of Vitamin A in Castrated Male Rats. By T. Moore, I. M. Sharman and R. J. Ward. (*Dunn Nutritional Laboratory, University of Cambridge, and Medical Research Council*)

Young male piebald rats, bred under conditions which ensured low initial reserves of vitamin A, were either castrated or left entire, and were kept for 27–29 days on a basal diet deficient in vitamin A, with supplements of 40 i.u. of synthetic vitamin A acetate daily. In previous work (Moore & Sharman, 1950) this level of dosing in male rats had been found to cause much higher concentrations of vitamin A in the kidneys than in the liver. Groups of five rats each were made up of (1) entire animals allowed food *ad lib.*, (2) castrated animals injected subcutaneously with testosterone propionate at the rate of 0·4 mg. daily and fed *ad lib.*, (3) castrated animals injected with testosterone, but given limited amounts of food, (4) castrated animals injected with oestradiol monobenzoate at the rate of 0·09 mg. daily, (5) castrated animals, without injections, fed *ad lib.* The food intakes of the animals given testosterone in group 3 were so adjusted that they grew at the same rate as those given oestradiol in group 4. At the end of the experiment the rats were killed, and vitamin A was estimated in their livers and kidneys by the antimony trichloride method.

From Table 1 it will be seen that high concentrations of vitamin A in the kidneys, typical of male animals under the experimental conditions chosen, were found in the entire rats which had an average of 37·4 i.u./g., in the castrated rats without injections which had 42·7 i.u., and in those given injections of testosterone and food *ad lib.* which had 35·7 i.u. In the castrated animals injected with oestradiol, in which growth was slow, the kidneys contained only 4·6 i.u./g., a value which might have been expected in female rats given the same allowance of vitamin A. In the group injected with testosterone and given limited amounts of food, the concentration of 18·7 i.u./g. was within the male range, although lower than in animals given similar injections but with unrestricted amounts of food.

In the liver the concentrations of vitamin A were highest and almost equal in the slowly growing groups, treated with oestradiol and testosterone

Table 1. *Vitamin A in kidneys and liver of rats given 40 i.u. of vitamin A daily (mean values)*

Group	Treatment	Mean wt. increase (g.)	Wt. of seminal vesicles (g.)	Kidneys		Liver		Total in liver + kidneys
				Total i.u.	i.u./g.	Total i.u.	i.u./g.	
1	Entire animal	109	0·575	75·0	37·4	118	10·5	193
2	Castrated + testosterone	97	0·705	76·1	35·7	183	19·9	259
3	Castrated + testosterone (limited food intake)	40	0·575	25·7	18·7	250	37·6	276
4	Castrated + oestradiol	40	0·064	7·5	4·6	261	35·2	269
5	Castrated (no injections)	98	0·004	71·9	42·7	230	26·0	302

with restricted food, at 35·2 and 37·6 i.u./g. respectively. The castrated animals without injections came next at 26·0 i.u., followed by the castrated animals with testosterone and food *ad lib.* at 19·9 i.u., and the entire animals at 10·5 i.u. The total amounts of vitamin A stored in the liver and kidneys combined were little different in all the castrated groups given hormones. Slightly higher amounts than in these three groups were found in the castrated animals without hormones, and slightly lower in the entire animals. The seminal vesicles, which were weighed to provide evidence of the efficiency of the hormone injections, were well developed in the entire rats and those injected with testosterone, rudimentary in castrated animals without injections, and very slightly developed in the animals injected with oestradiol.

These results indicate that the storage and distribution of vitamin A in the male rat are influenced by castration, and by the administration of sex hormones, particularly oestradiol. Apparently the influence of sex on vitamin A can only partially be explained as a secondary effect of its influence on growth.

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A Polysaccharide Isolated from *Corynebacterium diphtheriae*. By E. S. Holdsworth (introduced by F. C. Happold). (*Department of Biochemistry, University of Leeds*)

This communication is concerned with the nature and isolation of the polysaccharide which occurs in the cell wall of *Corynebacterium diphtheriae* P.W. 8. Preparations assumed to represent the cell wall were made in three ways. Freshly harvested washed cells were disintegrated by either (*a*) shaking with glass beads in a high-speed shaker, or (*b*) by repeated freezing and thawing, and the residues were well washed with 1 % sodium acetate solution. For large-scale work acetone-dried and ether-extracted cells were treated with 90 % phenol at 37° until no further material was extracted, and the insoluble residue was washed free from phenol with alcohol and ether. These three preparations appeared to give the same product, an insoluble polysaccharide-protein complex, yielding 24 % reducing sugars on hydrolysis. The carbohydrate and protein are firmly bound together, and the only method found to give quantitative yields of polysaccharide was to extract the complex with boiling aqueous picric acid solution. After purification this polysaccharide has been shown to have a mol. wt. 800–900 and to consist of D-galactose, D-mannose and D-arabinose. The protein portion of the complex differs from the phenol-soluble protein in that diamino pimelic acid, isolated by Work (1950) from *C. diphtheriae*, and hexosamine are present only in the complex of the cell wall.

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The Separation of Nucleotides by Ionophoresis. By R. M. S. Smellie and J. N. Davidson. (*Department of Biochemistry, University of Glasgow*)

The technique of ionophoresis (Consden, Gordon & Martin, 1946) as used for the separation of amino-acids on agar gel (Gordon, Keil, Sebesta, Knessl & Sorm, 1950) and paper (Durrum, 1950) provides a convenient method for the separation of the ribose mononucleotides; both agar gel and paper can be employed as transport media, but the latter has proved more satisfactory than the former. The nucleotides separate in the order uridylic acid, guanylic acid, adenylic acid and cytidylic acid in decreasing order of mobility, and their positions after separation can easily be located in ultraviolet light by the procedure of Holiday & Johnson (1949). The nucleotides can be recovered quantitatively on elution by the technique of Consden, Gordon & Martin (1947). The method can be used for the analysis of a purified ribonucleic acid, but it can also be employed with the mixture of ribonucleotides obtained by a modification of the Schmidt & Thannhauser (1945) separation scheme for tissue analysis. In this case uridylic acid is found to be preceded by a fast-moving spot containing a small amount of inorganic phosphate (derived from 'phosphoprotein') together with a larger proportion of organic phosphate.

In the determination of the specific activities of the protein-bound phosphorus fractions (including the nucleic acids) of tissues of animals which have received radioactive phosphorus, considerable difficulty may be experienced in the elimination of the last traces of contaminating inorganic radioactive-phosphate (Davidson, Frazer & Hutchison, 1951). The method of ionophoresis enables a satisfactory purification to be achieved. Thus when the liver tissue of rats which have received 0·1 μc.^{32}P/g. 2 hr. previously was fractionated and the ribonucleotides separated, the relative specific activities (counts/min./100 μg. P) were found to be as follows: adenylic acid, 300; cytidylic acid, 800; guanylic acid, 390; uridylic acid, 1280.

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Distribution of Vitamin A in the Organs of Marine Crustacea. By L. R. Fisher, S. K. Kon and S. Y. Thompson. (*National Institute for Research in Dairying, University of Reading*)

We have already reported that the preformed vitamin A of certain marine Crustacea is concentrated in the exoskeleton (Kon & Thompson, 1949) and that in *Euphausia superba* Dana, taken in the Antarctic from the stomach of a blue whale, two-thirds of the vitamin A was in the eyes (Batham, Fisher, Henry, Kon & Thompson, 1951).

Further work has shown that in four species, including the northern euphausiids, *Meganyctiphanes norvegica* (M. Sars) and *Thysanoessa raschii* (M. Sars),

Table 1. *Distribution of vitamin A in the organs of marine crustacea.*

Species	Date	Location	Mean weight of one specimen: Whole body (mg.)	Mean weight of one specimen: Eyes (mg.)	Mean vitamin A content of one specimen: Whole body (i.u.)	Mean vitamin A content of one specimen: Eyes (i.u.)	Vitamin A in eyes as % of that in body
Meganyctiphanes norvegica	21/11/50	Loch Fyne	60	2	1·1	1·0	99
M. norvegica	21/11/50	Loch Fyne	280	4	4·3	4·2	99
M. norvegica	21/11/50	Loch Fyne	400	6	14·7	14·3	98
M. norvegica	21/11/50	Loch Fyne	500	9	25·0	24·5	98
Thysanoessa raschii	10/1/51	Loch Fyne	60	1	5·5	5·4	98
Carcinus maenas	10/10/50	Burnham-on-Crouch	—	—	none	1·5	100
Eupagurus bernhardus	10/10/50	Burnham-on-Crouch	4100	10	none	0·5	100

the vitamin A is almost exclusively present in the eyes.

This location explains perhaps why free-swimming *Meganyctiphanes norvegica* was much richer in vitamin A than that taken from the stomach of the whale (Batham *et al.* 1951), since the vitamin might well be leached out of the eyes quite early in the process of digestion.

Table 1 shows the very marked increase in vitamin A content of *M. norvegica* with the size (age) of the animal. The four size groups were sorted from hauls taken at the same place and time. The concentration of vitamin A in the eyes of the euphausiids amounted to 32,000 i.u./g. lipids for the largest size of *M. norvegica* and to 57,000 i.u./g. lipids for *Thysanoessa raschii*.

This work was supported by a grant from the Development Commission. Grateful thanks are due to the staff of the Marine Station, Millport, and the Oyster Research Station, Burnham-on-Crouch, for facilities, assistance and advice.

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The Occurrence of Glycerylphosphorylethanolamine in Extracts of Liver and Yeast. By P. N. Campbell, D. H. Simmonds and T. S. Work. (*National Institute for Medical Research, Mill Hill, London, N.W.7*)

In a previous communication on chromatographic fractionation of water-soluble constituents of liver (Campbell, 1951), the presence of an unidentified acid-labile substance in liver extracts was reported. Further work has shown that this substance has the properties of glycerylphosphorylethanolamine. A small quantity of the substance has been isolated from pig liver.

Liver was homogenized, extracted and the dialysed extract passed through a column of the Zeo-Karb 225 as previously described. The ninhydrin-positive substances in the effluent were precipitated as their mercury salts (Neuberg & Kerb, 1912). The mercury salts were decomposed with hydrogen sulphide, and the product was fractionated on a paper column with amyl alcohol/pyridine/water as solvent. The procedure followed that described by Campbell, Mellanby & Work (1951). Three chromatographically pure components were isolated, taurine, ethanolamine phosphoric acid and the unknown. The unknown was isolated as a viscous oil which has not yet been crystallized, but the following evidence leaves little doubt as to its nature.

After hydrolysis with 5N-HCl for 16 hr. at 100° the only basic product was ethanolamine. The identity of the product was established by comparison on paper chromatograms with authentic samples of ethanolamine, 1-aminopropanol and 2-aminopropanol and by oxidation to glycine with acid $KMnO_4$ (Cooley, Ellis & Petrow, 1950). Ethanolamine was also formed by hydrolysis at 100° for 1 hr. with dilute $Ba(OH)_2$. This instability towards alkali suggested an ester rather than an amide linkage. The molybdate reagent of Hanes & Isherwood (1949) gave with the unknown on paper the strong blue spot characteristic of phosphorus. A determination of the N:P ratio by the methods of Harding & Maclean (1915) and of Allen (1940) indicated a ratio of 1:1. Paper chromatography, according to the method of Hanes & Isherwood (1949) using propanol/ammonia as solvent, of the unknown before and after hydrolysis with $Ba(OH)_2$ indicated the presence of glycerophosphoric acid only after hydrolysis. Acid hydrolysis resulted in the liberation of some inorganic phosphate from the glycerophosphoric acid. The presence of a glycol in the molecule was confirmed by the action of periodate and the subsequent positive reaction obtained with Schiff's reagent (Buchanan, Dekker & Long, 1950).

After acid hydrolysis of the unknown it was not possible to detect the presence of a fatty acid by steam distillation or by chromatography. Owing to the insolubility of the unknown in ether this result was not unexpected. It is therefore concluded that the properties of the unknown are best accounted for on the basis that it is glycerylphosphorylethanolamine (GPE). This substance has been previously described by Feulgen & Bersin (1939), who obtained it after hydrolysis of the plasmalogens of brain.

On a two-dimensional chromatogram using phenol/NH_3 and collidine/lutidine as solvents (Dent, 1948), GPE runs to a position very similar to that of citrulline, and it is, therefore, not possible to identify it in a complex chromatogram of a biological extract. However, citrulline reacts with $CuCO_3$ and GPE does not, so that by treating the paper with $CuCO_3$ as

described by Crumpler & Dent (1949), GPE is easily differentiated from citrulline and β-alanine. By this means its presence has been confirmed in fresh aqueous extracts of pig liver (after mercury precipitation). It has also been possible to detect GPE in extracts of foetal lamb liver, rabbit liver and ox liver (Walker, 1951) and also in extracts of brewer's yeast, but not in rat liver.

The ninhydrin spot due to GPE in pig-liver extracts is more pronounced than that due to ethanolaminephosphoric acid and is comparable to that due to taurine.

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Abnormal Mucopolysaccharide and 'Precollagen' in Vitamin C-deficient Skin Wounds. By J. R. G. Bradfield* and E. Kodicek. (*Department of Zoology and Dunn Nutritional Laboratory, University of Cambridge*)

Polysaccharide-protein complexes are important as connective tissue constituents in all classes of living organisms. Moreover, in man and the guinea pig, vitamin C is essential for the formation of these intercellular substances. In scorbutic wounds, either formation of connective tissue fails completely, or else abnormally thick, argyrophil strands of so-called 'precollagen' accumulate. The periodate-Schiff (PAS) method for staining polysaccharides (McManus, 1946; Hotchkiss, 1948) makes possible the study of polysaccharide behaviour during skin wound-healing in normal and scorbutic guinea pigs.

Twenty deficient animals were compared with twelve normals between January and September 1948, using the experimental procedure of Danielli, Fell & Kodicek (1945). The validity of the stain used will be considered elsewhere; it is highly probable that only polysaccharides react positively.

Normal and scorbutic wound sections differed markedly in their PAS staining. Ten-day-old normal wounds were packed with slender, parallel, interlacing fibrils, which stained faintly and homogeneously. During aggregation to thicker, mature collagen bundles (2–5 weeks), PAS-positive material almost disappeared. Ten-day scorbutic wounds contained numerous, thick, chaotically ramifying 'precollagen' strands, which stained intensely. Far from diminishing, this PAS-positive material accumulated, becoming very abundant in the oldest scorbutic wounds examined (16 days after operation or 26 days complete deficiency). This scorbutic PAS-positive material was concentrated in an irregular sheath round the 'precollagen', leaving a non-staining core. Collagenase removed the 'precollagen' core leaving only a shrivelled mass of sheath material, very strongly PAS-positive. With Azan stain, the core was blue (like typical collagen) and the sheath red, showing that the latter contained strongly acidic groups. Toluidine blue-metachromasia (Lison, 1935; Sylven, 1941) suggested that a little sulphated polysaccharide was present in scorbutic wounds (as in normal wounds), but gave much less marked contrast between the two than did the PAS, general-polysaccharide stain; hence sulphated polysaccharides are unlikely to account for all the abnormally abundant scorbutic polysaccharide. The latter was not markedly water-soluble (cf. Gersh & Catchpole, 1949) and removed neither by amylase, nor apparently by hyaluronidase. Finally, it is highly probable that the well known argyrophilia of scorbutic 'precollagen' and of the young, 'reticular' fibres in normal wounds (and elsewhere) is due to their polysaccharide content.

Evidently vitamin C, itself a carbohydrate derivative, has a profound influence on mucopolysaccharide metabolism, but whether direct or indirect, and whether affecting the kinds of polysaccharide produced, or the mucolytic enzymes for their disposal, is at present uncertain.

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The Assay of 'Strepogenin' Activity of Purified Proteins with *Lactobacillus casei*. By S. P. Mistry and E. Kodicek. (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*)

A satisfactory procedure for the assay of 'strepogenin' acitvity with *Lactobacillus casei* has been developed, based on a modified medium used for the determination of pteroylglutamic acid (Kodicek & Mistry, 1949). A preparation of crystalline bovine insulin, hydrolysed with conc. HCl for 3 hr. at 37°, was used as the 'strepogenin' standard. For the assay, a relatively small inoculum was found to give the best results. The growth responses obtained from at least three dose levels were measured turbidimetrically after 18 hr. incubation at 37°. The logarithm of the dose plotted against the dry weight of bacteria gave a linear response. The method was applied to the assay of 'strepogenin' in highly purified proteins, in liver and yeast preparations, and the results will be discussed (Table 1). Insulins from various species differed in their activity. In some materials, such as liver preparations and virus proteins, the dose-response curve had a steeper slope than that of acid-hydrolysed insulin, indicating that the former contain factor(s) considerably more potent than the insulin standard.

The peptide(s) responsible for the 'strepogenin' activity were shown to be an integral part of the protein molecule and not associated with an impurity, such as the hyperglycaemic-glycogenolytic factor present in commercial preparations of crystalline insulins. The possibility exists that the 'strepogenin' activity is a characteristic of several peptides. Glutamine, aspartic acid and asparagine showed a negligible strepogenin-like activity which, however, did not increase with increasing concentrations. In contrast to this, glutamic acid and glutathione were inactive.

Table 1. *'Strepogenin' activity of purified proteins related to the activity of acid-hydrolysed insulin, bovine, Boots*

Material*	Relative 'strepogenin' activity (%)
Insulin, pig, Boots	412
Insulin, sheep, Boots	400
Insulin, bovine, Boots, recrystallized	95
Insulin, bovine, Boots, hydrolysed with chymotrypsin	103
Insulin, Novo, free of hyperglycaemic-glycogenolytic factor	90
Pepsin, Armour, 1:10,000 potency	39
Pepsin, Armour, recryst., 1:60,000	160†
Chymotrypsin, Armour	153†
Trypsin, Armour	48
Turnip yellow mosaic virus	86†
Tobacco mosaic virus	56†
Myosin, rabbit	104†
Lactoglobulin, cryst.	49†
Plasma albumin, bovine, Armour	45
Egg albumin	19
Crude protein from degraded wool	13
Globin, horse	10
Globin, human	9
Globin, cow	5
Haemoglobin, bovine, Armour	5
Pea globulin	4
Edestin	2
Gelatin	1
Salmin sulphate, British Drug Houses Ltd.	0
Casein, vitamin-free Labco	5
Casein, vitamin-free, Glaxo	2
Hepamino, proteolysed liver powder, Evans (unhydrolysed)	20†
Liver fraction L, Wilson (unhydrolysed)	8
Marmite, yeast extract (unhydrolysed)	6

* Except where mentioned all materials were hydrolysed with conc. HCl for 3 hr. at 37° and tested at three levels.

† Values denote activity not strictly comparable to the insulin standard.

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The Action of Acid Phosphate on the Anion Permeability of Horse Erythrocytes. By F. A. HOLTON (introduced by D. KEILIN). (*Molteno Institute, University of Cambridge*)

In 1941, Keilin & Mann described an optical method of studying the penetration of erythrocytes by bicarbonate ions and the intracellular activity of carbonic anhydrase. This method consists of converting the haemoglobin inside the cell to methaemoglobin and observing the transformation of the methaemoglobin from the acid to the alkaline form by the entering bicarbonate ions. During this work they noticed that this transformation did not occur if the methaemoglobin cells had been washed in acid phosphate and interpreted this as an effect on the permeability of the cells. Similar observations by Keilin & Hartree (1946) were interpreted as showing that phosphate, phthalate and citrate buffers of pH 6 also 'block' the anion permeability of such cells.

These optical experiments have been repeated together with measurements of changes in the opacity of cell suspensions and chemical analyses of cells 'blocked' by phosphate buffer pH 6. There is a consistent relationship between the completeness of the 'block' and (*a*) the amount of phosphate that has penetrated the cells, and (*b*) the amount of chloride that has left the cells. It is therefore suggested that the effect of acid buffers is due to the replacement within the cell of rapidly diffusing chloride ions by slowly diffusing acidic ions, rather than to decreased cell permeability. Since bicarbonate ions can only enter the cell in exchange for an equivalent amount of intracellular anions, such replacement greatly reduces its rate of penetration.

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Fermentations with Mixed Strains of *Aspergillus niger*. By J. L. YUILL. (*John and E. Sturge (Citric) Ltd., Birmingham* 15)

The group species *Aspergillus niger* is well known to comprise a great number of different strains varying both morphologically and biochemically. In particular, the capacity to ferment sugar to citric acid varies enormously from strain to strain.

It has been reported (Palei, 1935) that 'common cultures of two strains of *A. niger* in one dish show mutual effects ranging from growth inhibition to no apparent influence'.

In the experiments now reported it was found that when two different, unrelated, high-acid strains of *A. niger* were grown in mixed culture on molasses solution, the yield of acid produced was much less than that of either strain grown separately. When two unrelated low-acid strains were grown in mixed culture, the acid yield was greater than that of either of the moulds alone. In both cases the weight of the mycelium of the mixed culture was considerably greater than that of either alone. The coupling of a high-acid and a low-acid (unrelated strain also led to the elaboration of a heavier mycelium, and the acid yield was intermediate between those of the separate moulds.

When two strains related as mutant and parent or as different mutants of the same parent were grown in mixed culture, the acid yield was not depressed and the weight of mycelium was not increased.

Unrelated strains of *A. niger* no doubt differ from each other in many more ways than do, for example, colour-mutants of the same mould, and in some way the differences between the above unrelated strains have brought about a stimulation of mycelial growth.

It has been shown (Gossop, Yuill & Yuill, 1940) that the hyphae of strains of Aspergilli possessing mutant relationship form anastomoses freely in mixed culture, giving rise to heterokaryotic mycelium and spore heads. Whether this also can hold good for 'unrelated' strains is being investigated. Again the cause of stimulation may reside in substances which can pass through the cell walls of the hyphae.

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The Determination of Glucosamine by Alkaline Decomposition. By M. V. TRACEY. (*Rothamsted Experimental Station, Harpenden, Herts*)

Glucosamine is rapidly decomposed by alkaline solutions with the evolution of ammonia. Distillation in a Markham (1942) still of glucosamine solutions in the presence of saturated Na_3PO_4 solution leads to quantitative recovery of ammonia from the glucosamine. Under these conditions glucose gives rise to volatile products that interfere with the estimation of ammonia by Nesslerization. The addition of sodium borate to the Na_3PO_4 solution stops the appearance of these products in the distillate. Glutamine to a marked extent and asparagine to a lesser extent decompose under these conditions. Decomposition of urea is negligible. Since glucosamine is relatively stable in acid solutions preliminary boiling of solutions containing asparagine or glutamine for 3 hr. in N-H_2SO_4 completely hydrolyses them without affecting glucosamine. Free ammonia or ammonia derived from the acid hydrolysis of glutamine, asparagine, or urea may be estimated by microdiffusion in a Conway dish at room temperature. Under these conditions glucosamine is stable. Glucosamine may therefore be estimated in the presence of large amounts of amino-acids and sugars by distillation of an acid-hydrolysed solution in a Markham still, the amount of volatile nitrogen being corrected by subtraction of that found by microdiffusion at room temperature. The sensitivity of the method is about one-eighth of that of the method used for the determination of ammonia. A flask for microdiffusion at 100° has been made and is of value for solutions in which frothing makes distillation in the Markham still tedious.

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A Third Amino-acid Amide in Peanut Plants (*Arachis hypogaea*). By J. DONE and L. FOWDEN. (*Medical Research Council Laboratories, Holly Hill, Hampstead, London, N.W.3*)

Exudates from the cut surfaces of turgid stems of peanut plants were examined by the methods of paper chromatography. All samples contained, as the main ninhydrin-reacting component, a substance (I) which formed an orange-brown spot (R_F 0·66 in phenol-NH_3). After treatment of the exudate with N-HCl for 3 hr. at 100°, substance I could no longer be detected, but another substance (II), which gave a yellow-brown spot with ninhydrin (R_F 0·30 in phenol-NH_3), was observed. During hydrolysis, NH_3-N equivalent to 40% of the total N was produced. Autolysis of homogenized leaf tissue for 3 hr. at 37° also gave II.

The easily water-soluble substance I was isolated from exudates by means of Zeo-Karb 215 (Partridge & Westall, 1949), and recrystallized from aqueous ethanol. (Found: C, 46·0; H, 6·5; N, 17·3. Calc. for $C_6H_{10}N_2O_3$: C, 45·6; H, 6·3; N, 17·7%.) Treatment with N-NaOH released half of the N as NH_3.

Substance II was isolated from autolysed plant tissues by means of De-Acidite B (Partridge & Brimley, 1949), and recrystallized from water. (Found: C, 44·1; H, 5·7; N, 8·60; amino-N (van Slyke), 8·43%. Calc. for $C_6H_9NO_4$: C, 45·2; H, 5·7; N, 8·80%.) Evidence for the presence of a free COOH group was obtained by tritation.

These data suggest that II is an acid derived from an amide I.

Treatment of II in aqueous suspension with O_3 produced a substance indistinguishable from aspartic acid by paper chromatographic methods. Its isolation was effected through the Cu-salt, and fumaric acid was obtained after exhaustive methylation (Braunstein, Nemchinskaya & Vilenkina, 1947). Oxidation of II in 10% Na_2CO_3 with powdered $KMnO_4$ gave oxalic acid and a substance which behaved in a similar manner to aspartic acid on paper chromatograms. Oxalic acid was not detected during or after ozonolysis.

An aqueous suspension of substance II was hydrogenated in the presence of palladium on carbon. The H_2 absorbed corresponded to 1·04 mol. for the reaction $C_6H_9NO_4 \rightarrow C_6H_{11}NO_4$. The product (III) was easily soluble in water and was recrystallized from aqueous acetone. (Found: C, 44·7; H, 6·8; N, 9·14. Calc. for $C_6H_{11}NO_4$: C, 44·7; H, 6·8; N, 8·70%.) Equiv. wt. by titration with 0·103 N-NaOH = 159. (Equiv. wt. of monobasic $C_6H_{11}NO_4 = 161$.) It gave a purple spot with ninhydrin. (R_F 0·42 in phenol-NH_4.) No optical activity was detected.

Since aspartic acid is produced by ozonolysis, (II) would appear to contain $COOH.CH(NH_2)CH_2.C=$, and since a titratable COOH is also present, possible formulae are

$$COOH.CH(NH_2)CH_2.CH=CH.COOH \text{ (V)}$$

and

$$COOH.CH(NH_2).CH_2.C(=CH_2).COOH \text{ (VI)}.$$

The corresponding saturated compounds are α-amino adipic and γ-methylglutamic acids, respectively. Substance (III) could be separated from DL-α-amine-adipic acid by paper chromatography. No γ-methyl-glutamic acid was available.

Compound I was detected in various tissues of young and mature peanut plants. It could not be found in a commercial arachin hydrolysate or in immature or ripe seeds, but it appeared after germination. Considerable amounts of glutamine and asparagine were present in tissue homogenates, but only traces were detected in the exudates. The compound I has not been found in other plant species examined.

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DEMONSTRATIONS

An Apparatus for Micro-filtration. By A. L. Latner. (*Department of Pathology, Royal Victoria Infirmary, Newcastle upon Tyne*)

The apparatus has proved useful in recrystallizing small quantities of sterol compounds. It comprises a micro-filter and a micro-beaker. The former consists of a filter stick with a spherical head, which is lightly ground into the lower end of a short glass tube with an expanded top. These two have lugs on their outer surface and are held together by short lengths of wire attached to small rubber bands. The wire portion is lowermost in order to prevent contamination of the organic solvent filtrate with rubber. The micro-beaker is made from capillary tubing, which has been expanded at one end. The capillary stem passes through a rubber bung to which is attached a thick rubber band stretched over the capillary end.

Material for crystallization is dissolved in a few drops of hot solvent and the solution transferred with a teat-end capillary pipette to the expanded end of the micro-beaker, which is immediately inverted into a thick-walled test-tube. Surface tension holds the liquid in place, and crystallization occurs in the region of the surface. If necessary, the beaker assembly can be placed in a refrigerator. The inverted beaker is now held inside the micro-filter just above the spherical head of the filter stick. Gentle suction is applied by the water pump and the rubber band slipped off the capillary end. Almost complete transfer of the material to be filtered can be assured by gently blowing through the capillary. The rubber band is replaced and the micro-beaker washed with a drop or two of cold solvent, which is then filtered off.

Before using the beaker, it is advisable to fill the upper end of the capillary lumen with cold solvent.

Filter-paper Chromatography of Phosphate Esters. By D. G. Walker and F. L. Warren. (*Department of Biochemistry, University College, London, W.C.*1)

The method used is based on that of Hanes & Isherwood (1949). The necessity for and the methods of pre-washing the filter paper will be demonstrated. Paper used with acid solvents, e.g. *tert.*-amyl alcohol/water/toluene-*p*-sulphonic acid (water-poor phase), is improved by previous treatment with H_2S gas.

The main difficulty in applying the method to natural extracts is interference by ions in the extract itself. The addition of a trace of a chelating agent such as ethylenediamine tetra-acetic acid ('Versene') has proved effective. In the presence of this reagent the phosphoric esters tend to run more slowly, and it is necessary to add an equivalent amount of 'Versene' to the control solutions. The order in which the esters move down the paper is not, however, affected.

A new spraying reagent, specific for fructose esters (fructose-6-phosphate and fructose-1:6-diphosphate) has proved useful in interpreting chromatograms of tissue extracts. The spraying solution (10 ml.) contains: naphthoresorcinol (4·75 ml. 0·2% w/v in ethanol), trichloracetic acid (4·75 ml. 2% w/v) and perchloric acid (0·5 ml. 60% w/w). The sprayed papers are heated for 8 min. at 85° when the fructose esters appear as cherry-red spots on a pale-mauve background.

Extension of the method to two-way chromatograms facilitates the complete separation of sugar phosphate esters and related compounds when using solutions of pure compounds. The main limitation of the technique is that there is a definite maximum in the amount of material which can be put on the filter paper without serious defects arising, especially when tissue extracts are examined.

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Application of Polarography in a Study of Cystinuria. By H. HARRIS and F. L. WARREN. (*Department of Biochemistry and the Galton Laboratory, University College, London, W.C.*1)

Cystine can be estimated polarographically from the height of the characteristic wave obtained in ammonia-ammonium chloride buffer solutions containing a divalent cobalt salt. The sensitivity of the method is high and the cystine can usually be estimated at dilutions at which the polarographic effect of urinary protein becomes negligible. For example, in normal urines dilutions of 1:50 to 1:100 give measurable waves, and in cystinuric urines dilutions ten times as great can be employed.

Reed (1942) reported daily excretions of 40–80 mg. of free cystine in normal subjects and also found that mild hydrolysis liberated a further quantity of cystine in about the same amount. These findings have been confirmed in the present work.

A survey is being made of the cystine content of urines from cystinurics, from their relatives, and from a random sample of the population.

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Improved Chromatography Fraction Collector. By E. M. CROOK and S. P. DATTA. (*Department of Biochemistry, University College, London, W.C.*1)

The capacity of the fraction collector is 150 tubes with a maximum volume of 50 ml./tube. Metering of fractions is based on the use of a proximity switch and electromagnetic valves. Advantages of the design are: (1) improved accuracy of volume measurement ($\pm$ 0·1 ml.), (2) sets of matched collection tubes are not required, (3) no loss of column effluent between fractions, (4) ability to fractionate wide range of flow rates without loss of accuracy, (5) solvent may be changed without special adjustment, (6) provision of adequate safety devices to guard against power or component failures.

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **296th Meeting** *of the Biochemical Society was held in the Department of Physiology, University of Bristol, Bristol* 8, *on Friday,* 6 *April* 1951, *when the following papers were read:*

COMMUNICATIONS

The Minor Carotenoid Components of the Fungus *Phycomyces blakesleeanus*. By T. W. Goodwin. (*Department of Biochemistry, University of Liverpool*)

It has previously been shown that β-carotene is by far the major carotenoid present in *Phycomyces* (Schopfer, 1935; Karrer & Krause-Voith, 1947; Bernhard & Albrecht, 1948; Garton, Goodwin & Lijinsky, 1951); α-carotene occurs in small amounts and lycopene has been reported in traces (Bernhard & Albrecht, 1948). Chromatographic examination of the lipid extracts of large cultures of *Phycomyces* revealed the presence of a small coloured zone adsorbed on Al_2O_3 just above β-carotene. This fraction has been separated into four components which have been unequivocally identified as γ-carotene, ζ-carotene, neurosporene and lycopene. A small brownish-yellow band occasionally encountered just above lycopene has no characteristic properties and is probably a mixture of oxidized carotenes. Recently, Schopfer & Grob (1950) remarked on the presence of five carotenoids in *Phycomyces*, but have not yet discussed their identification. In about 5% of the cultures examined, a bright-red pigment is present which is very strongly adsorbed on alumina and appears to be a quinonoid compound. The partly saturated carotenoids phytofluene and phytoene have also been identified in *Phycomyces*; this is the first time that phytoene has been reported in a fungus. A material with an absorption maximum at about 255 mμ., closely associated with phytoene on chromatography, is also present, but has not yet been identified; the presence of tetrahydrophytoene is still to be confirmed.

Variations in cultural conditions which alter the total amount of carotenes produced (see Garton *et al.* 1951) do not alter significantly the relative amounts of the constituents. The relative amounts produced by the (+) and (−) strains are also the same.

Diphenylamine, which inhibits carotenogenesis in *Mycobacterium phlei* (Turian, 1950), when added to the culture medium at a level of 1/40,000, has little effect on growth and lipid production of *Phycomyces*, but it specifically inhibits the synthesis of β-carotene, whilst stimulating the synthesis of phytofluene and phytoene. Of the minor components, lycopene and α- and γ-carotene production is inhibited, whilst that of neurosporene and ζ-carotene is increased. The bearing of these observations on schemes which have been put forward to explain the mechanism of carotenogenesis was discussed.

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The Metabolism of Some Benzoxazoles in the Rabbit. By H. G. Bray, R. C. Clowes and W. V. Thorpe. (*Department of Physiology, Medical School, University of Birmingham*)

The fate of benzoxazole, 2-methylbenzoxazole, 2-phenylbenzoxazole and benzoxazolone in the rabbit has been investigated. The oxazole ring in benzoxazole and in 2-methylbenzoxazole appears to be readily broken *in vivo*, and *o*-aminophenol and some of its derivatives have been identified as metabolites in amounts accounting for practically the whole of the dose. This has involved a study of the metabolism of *o*-aminophenol and its acyl derivatives. There appears to be no fission of the oxazole ring in 2-phenylbenzoxazole, since no amino-compounds could be detected in the hydrolysed urines of rabbits dosed with this compound. Hydroxylation accounted for about 60% of the dose and a hydroxy-

phenylbenzoxazole, probably 2-(3′-hydroxyphenyl)-benzoxazole, and its glucuronide were isolated. Hydroxylation corresponding to about 75% of the dose occurred with benzoxazolone and there was no evidence of fission of the oxazole ring. The finding of Gressly & Nencki (1890, 1891) of a hydroxybenzoxazolone was confirmed and a glucuronide was isolated.

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The 'Lewis' (Le^a) Human Blood-Group Substance. By E. F. Annison and W. T. J. Morgan. (*Lister Institute of Preventive Medicine, London, S.W.* 1)

A new human blood-group character called 'Lewis' was discovered recently (Mourant, 1946). The erythrocytes of about 20% of adult English people are agglutinated by anti-'Lewis' sera and the agglutination is independent of other known blood group characters (ABO, MN, Rh, P, etc.). Two 'Lewis' genes are now recognized and are designated by the symbols Le^a and Le^b; the corresponding antibodies to the gene products by anti-Le^a and anti-Le^b. It has been found that the secretions of individuals who are 'Lewis-positive', that is, of persons whose erythrocytes are agglutinated by anti-Le^a serum, do not contain the A, B and H (so-called O) substances which might be expected according to the blood group of the individual. The secretions from 'Lewis'-positive persons contain Le^a substance and the isolation and characterization of this substance, which is responsible for the Le^a character of human erythrocytes and secretions, is now reported.

The Le^a substance was isolated from human ovarian cyst fluids by methods similar to those already described (cf. Aminoff, Morgan & Watkins, 1950) and identified as a mucoid which possesses intense Le^a activity. It is similar in chemical and physical properties to that responsible for blood-group A activity. The Le^a substance isolated is essentially homogeneous according to the results of electrophoretic examination at pH 4 and 8, and its behaviour in the Svedberg ultracentrifuge indicates that although it is somewhat polydisperse, at a concentration of 0·5% the mean sedimentation constant S_{20}, is $6{\cdot}7 \times 10^{13}$, and the particle weight is of the order of 270,000.* The substance contains 5·0% N, about 10% acetyl, and no significant amount of P or S. It possesses a relative viscosity, η_r, of 1·36 (0·5% in 0·85% NaCl) and shows no characteristic absorption over the wavelength range 220–300 mμ. The acid hydrolysis products of the Le^a substance contain 57% reducing substances, expressed as glucose, and 32% hexosamine, as base. The α-amino-acid N represents about 37% and the α-amino N 92% of the total N. L-Fucose, 13% (identified as diphenylhydrazone, m.p. 201° uncorr.), D-galactose (identified as methylphenylhydrazone, m.p. 191° uncorr.), D-glucosamine, D-chondrosamine and eleven amino-acids have been identified as components of the Le^a substance. The glucosamine/chondrosamine ratio, determined by the isolation of these amino sugars as DNP derivatives (Annison, James & Morgan, 1950) is 3:1.

* Determined by Dr R. A. Kekwick and Dr A. G. Ogston.

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Studies on the Origin of Ethanolaminephosphoric Acid in Brain Tissue. By R. M. C. Dawson and G. B. Ansell. (*Neuropsychiatric Research Centre, Whitchurch Hospital, Cardiff*)

Ethanolaminephosphoric acid was identified by a chromatographic procedure as a component of the acid-soluble P fraction of rat brain. The acid was present in extracts prepared from brain tissue which had been fixed *in situ* with liquid oxygen; and this indicated that it was not formed by post-mortem enzymic activity.

The uptake of radioactive phosphorus, ^{32}P, into the ethanolaminephosphoric acid of rat brain was measured. After allowing a period of 3 hr. for

isotopic exchange in the intact animal, the ethanolaminephosphoric acid P isolated from the brain had a specific activity which was 50–70% of the specific activity of the corresponding inorganic and labile P fraction. Isolation of the ether-soluble phospholipins showed that the P of this fraction had a specific activity 3 hr. after ^{32}P injection, which was $\frac{1}{15}$ to $\frac{1}{19}$ of that of the ethanolaminephosphoric acid. This suggests that the ethanolaminephosphoric acid of rat brain does not arise by the enzymic breakdown of phosphatidyl ethanolamine present in the brain. It is known that the ether-soluble phospholipins of rat brain contain about 40% of phosphatidyl ethanolamine (Artom, 1945), and even if the turnover of ^{32}P were selectively located in the ethanolamine-containing phospholipin, its specific activity would still be many times less than that of the corresponding ethanolaminephosphoric acid.

Experiments showed that synthesis of radioactive ethanolaminephosphoric acid occurred when isolated rat-brain tissue was incubated aerobically in glucose-bicarbonate-Ringer solution containing radioactive phosphate. This formation of the acid *in vitro* appeared to be independent of the catabolism of phosphatidyl ethanolamine and a simple phosphorylation of ethanolamine was indicated.

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Measurement of Thyroid Activity and Its Relation to the Basal Metabolic Rate. By C. P. Haigh, Jean M. Reiss and M. Reiss. (*Endocrinological Research Department, Bristol Mental Hospitals*)

A method is described of estimating thyroid function using carrier-free radioactive iodine which provides an arbitrary numerical scale of different states of thyroid function. The method appears to be particularly suitable for identifying border-line cases of thyroid dysfunction which are not easily resolved clinically, nor by other radioactive tracer methods. The technique described involves measurement of the rate of iodine uptake during the first hour, and measurement of the iodine excreted during 60 hr., both from the time of administration of the radioactive iodine. The ratio of the rate of iodine uptake to the total iodine excreted is taken as the measure of thyroid activity. The experimental errors will be discussed and the results on over 500 mental patients given in the form of separate histograms for male and female patients, since, on the average, women show greater thyroid activities than men. This is also true of normal control subjects. Arbitrary limits of normality have been defined on the basis of these results.

In many cases, the B.M.R. of the patient was also determined using the spirometric method, and whilst usually there was a reasonable correlation between the two results, a group of subjects has been found in which an increased thyroid activity, as measured by the radioactive iodine technique, was accompanied by normal and even subnormal B.M.R. and vice versa. These paradoxical results suggested the existence of a disturbance in the sensitivity of the body tissues to thyroid hormone. The extent to which states of decreased sensitivity could be the pathogenetic cause of the development of thyroid hyperactivity will be discussed.

Hydrolytic Enzymes of Rat Adrenal Cortex. By J. J. Gordon. (*Endocrinological Research Department, Bristol Mental Hospitals*)

Histological methods have indicated that the adrenal cortex of hypophysectomized animals may undergo important changes in lipid content (indicating possible changes in lipase activity) (Reiss, Balint, Oestreicher & Aronson, 1936), and also in alkaline phosphatase activity (Dempsey, Greep & Deane, 1949). In view of these effects, it was of interest to investigate such hydrolytic enzymes chemically.

Preliminary experiments have been carried out to characterize these systems, and to develop methods for their assay in small amounts of adrenal cortical tissue. The demedullated adrenals of white rats have been successfully employed for this purpose. Homogenates prepared from them showed strong lipase activity when tributyrin or triacetin were employed as substrates, but showed much lower activity with diacetin or monoacetin as substrates. Of a series of simple aliphatic esters tested, only *n*-butyl propionate showed a rate of hydrolysis higher than tributyrin. Tributyrin hydrolysis was inhibited appreciably by the sodium salts of cholic, deoxycholic and taurocholic acids, and by NaF, but was only slightly inhibited by benzaldehyde.

Homogenates of demedullated rat adrenals also showed strong phosphatase activities characterized by pH and substrate concentration optima, when

sodium α-glycerophosphate, sodium pyrophosphate, or adenosinetriphosphate were used as substrates. Mg^{++} had no effect on the acid phosphomonoesterase, but it appreciably activated the alkaline phosphomonoesterase, and was essential for the activity of inorganic pyrophosphatase. Adenosinetriphosphatase (ATP-ase) was activated to a greater extent by Mg^{++} than by Ca^{++}. Inorganic pyrophosphatase and ATP-ase were also activated by cysteine.

The extent of hydrolysis initially varied directly with time and enzyme concentration, and the levels of activity may be accurately estimated in amounts of tissue obtainable from the adrenals of single rats.

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The Nutrition of the Dried Fruit Beetle (*Carpophilus hemipterus*). By G. O. Stride (introduced by E. W. Yemm). (*Department of Zoology, University of Bristol*)

The beetle, *Carpophilus hemipterus*, is frequently a pest of dried fruit and other stored products. If reared from the egg under sterile conditions the larvae grow rapidly on a basic mixture of dried yeast, casein, glucose, cholesterol and McCollum salt mixture (McCollum, Rask & Becker, 1928). A solution of Imperial Chemical Industries 'Cellophas WFZ' was incorporated with the basic mixture to provide a suitable consistency and the necessary water.

The high proportion of yeast necessary for growth suggests that fungi are an important factor in the diet under natural conditions. Attempts were therefore made to grow the larvae on dried fruit under sterile conditions. The larvae died. Normal growth could be obtained by: (1) mixing dried yeast with the dried fruit before sterilization; (2) inoculating the sterilized dried fruit with living yeasts or moulds. Rapid growth under natural conditions therefore seems to be the result of either selected feeding on fungi present on the dried fruit or the activities of a microfauna possibly acquired by the larvae.

The extraction of dried yeast with petroleum ether reduces its growth-promoting properties. They can be almost completely restored by adding cholesterol or ergosterol.

Of the vitamins of the B group, at least riboflavin, nicotinic acid and pantothenic acid, are essential for survival.

Yeast appears to contain a further factor which is essential for growth. This factor also occurs in various moulds. It appears to be insoluble or only slightly soluble in water, alcohols or petroleum ether. It is not tryptophan, thymine, yeast nucleic acid or biotin.

Previous workers (Goldberg, De Meillon & Lavoipierre, 1945; Begg & Robertson, 1950) have shown that a water-insoluble factor present in yeast is important in the nutrition of both *Aedes aegypti* and *Drosophila melanogaster*.

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The Cytoplasmic and Chloroplastic Proteins of Barley. By E. W. Yemm. (*Department of Botany, University of Bristol*)

Tissue proteins, separated from mature leaves and seedlings of barley, have been analysed in order to gain further information concerning the synthesis and inter-conversion of amino-acids during the growth and development of the plants.

Preparations of the proteins were made, using the methods developed by Chibnall (1939) and Neish (1939), as follows: (1) whole-tissue protein of leaves, including cytoplasmic and chloroplastic fractions; (2) cytoplasmic proteins from leaves; (3) whole-tissue proteins from young seedlings.

Estimates of the constituent amino-acids of these preparations were obtained mainly by means of microbiological assay following the general methods of Henderson & Snell (1948). By this means, 94–96% of the total nitrogen in the hydrolysates was accounted for by the amino-acids, glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, serine, threonine, cystine, methionine, lysine, histidine, arginine, proline, aspartic and glutamic acids and amide N.

A comparison of the different preparations of tissue

protein indicates a high degree of uniformity in the proportions of their constituent amino-acids. Appreciable differences were found only in their lysine content, the seedling and cytoplasmic preparations containing a higher proportion of this amino-acid. There is some evidence that the uniformity in the composition of the proteins may be paralleled in their physical properties; Wildman & Bonner (1947) have shown that 70–80% of tissue proteins of spinach leaves are electrophoretically homogeneous.

With regard to the synthesis of protein in the plant, the results indicate that the requirements of amino-acids for the synthesis of both chloroplastic and cytoplasmic protein are relatively uniform, not only in mature leaves but also in the young seedlings.

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Amino-Acid Interconversion during the Germination of Barley Grains. By B. F. Folkes (introduced by E. W. Yemm). (*Department of Botany, University of Bristol*)

The nature and extent of the amino-acid interconversions involved in the breakdown of the seed-storage proteins and the synthesis of the embryo-tissue proteins during germination, may be gauged by comparing the amino-acid contents of the two sets of proteins (see Chibnall, 1939; Yemm, 1949). This has been done for barley; the endosperm proteins have been prepared and their analyses by microbiological assay may be compared with those of the tissue proteins already presented. The two main endosperm proteins, hordein and hordenin, are richer than the tissue proteins in amide, glutamic acid and proline, and deficient in the basic amino-acids. Over 60% of the hordein N is in glutamine and proline residues. The albumin is almost identical in composition with the tissue proteins, and the globulin is fairly similar.

A more precise estimate of inter-conversions has been made by growing barley under controlled conditions in sand moistened with a mineral culture solution lacking in nitrogen, and taking samples at intervals for analysis into the protein and non-protein fractions. The endosperms were analysed by modifications of the methods of Bishop (1928, 1929). The embryo-tissue protein was estimated from the total N of the water- and acetone-insoluble residue. The contents of some amino-acids were estimated in the non-protein fractions by indirect methods and microbiological assay.

From these data it is possible to calculate the changes in content of each of the amino-acids during germination. Synthesis of aspartic acid, alanine, lysine, arginine, purines and chlorophyll, and to a lesser extent of glycine, isoleucine, tryptophan and threonine, are uniformly observed during germination. In these syntheses, 90% of the nitrogen involved is derived from amide, glutamic acid and proline. Decreases in phenylalanine and serine are also observed. As proline can be oxidized to glutamic acid by an animal enzyme system (Taggart & Krakaur, 1949), the central position of glutamic acid and glutamine in protein synthesis is strongly underlined by these results.

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Synthesis of Amino-Acids in Young Roots of Barley. By A. J. Willis (introduced by E. W. Yemm). (*Department of Botany, University of Bristol*)

Nitrogen assimilation in barley has been investigated by the use of roots from 10 day-old seedlings grown under controlled conditions of high light intensity in mineral culture solution deficient in nitrogen. The excised roots rapidly absorb and metabolize ammonium salts, nitrates and nitrites; the course of nitrogen assimilation from these sources has been followed by analysing the roots after various periods of treatment.

The most important changes involve sugars and nitrogenous constituents, chiefly the soluble amides. Glutamine is the primary synthetic product, and

this has been identified and estimated by hydrolysis at pH 6·5, by the use of *Clostridium welchii* (Krebs, 1948), and by chromatographic methods. Asparagine is formed more slowly and in smaller quantities than glutamine, but is more important in the assimilation of nitrate than ammonia. The large increases in the α-amino N content of the tissues are entirely attributable to those of glutamine and asparagine, except when nitrates are applied, when there are indications of a small synthesis of other amino-acids. Protein synthesis is only slight; proteolysis occurs after the roots have been excised for about 20 hr.

Additional evidence relating to the changes in nitrogenous constituents has been gained by the use of the stable isotope ^{15}N. Ammonium phosphate containing about 30 atom % excess ^{15}N has been applied to the root systems and the N compounds of the latter fractionated. The isotope was incorporated into all N fractions analysed, the highest content being found in the amide group of glutamine (with the exception of free ammonia). The amide group of asparagine and the α-amino N also showed high ^{15}N contents, indicating *de novo* synthesis of both glutamine and asparagine. There is considerable incorporation of ^{15}N into the protein, but no evidence of an overall synthesis. The amide groups of the protein contain a higher proportion of the isotope than the other nitrogen; it seems probable that these amide groups play an important part in exchange reactions associated with protein metabolism.

Glutamine formation during ammonia assimilation is intimately associated with carbohydrate metabolism. The carbon skeleton of glutamine arises largely from sugars, and glutamine synthesis is coupled with greatly increased respiration rates. Glutamine may act as an important link in the transference of energy from oxidative processes to protein synthesis.

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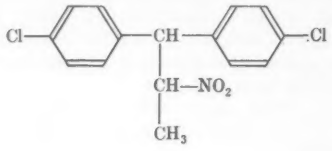
The Insecticidal Action of DDT. By E. J. Skerrett, A. Stringer and D. Woodcock. (*Research Station, Long Ashton, Bristol*)

Since the discovery of the outstanding insecticidal activity of 1:1:1-trichloro-2:2-di-*p*-chlorophenylethane (DDT), two general theories depending ultimately on the classical Overton-Meyer theory relating lipoid solubility to physiological activity have been put forward. There is considerable evidence both against the toxophore concept of Läuger, Martin & Müller (1944) and the dehydro-



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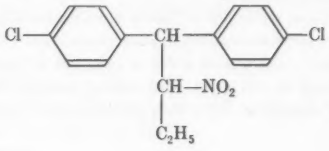

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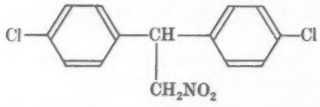

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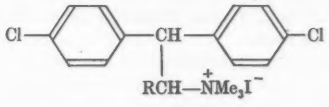

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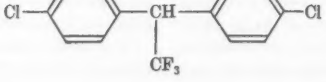

V

chlorination hypothesis of Martin & Wain (1944). More recent suggestions place greater emphasis on the spatial relationship of different parts of the molecule, and various workers have reached the conclusion that the most toxic analogues are very similar to DDT both in shape and weight (Busvine, 1946; Prill, Synerholm & Hartzell, 1946).

In view of the early references to the necessity for the presence of the three β-chlorine atoms for optimal toxicity, it was decided to investigate more fully their role. If the toxicity of DDT is due to its structural resemblance to some essential insect metabolite, then isosteric compounds should presumably act similarly, and replacement of the three β-chlorine atoms by spatially similar atoms or groups of atoms should not destroy activity.

A number of compounds involving the substitution of hydroxyl or methyl groups for the chlorine atoms in the DDT molecule have been synthesized (Skerrett & Woodcock, 1950). Tested as contact insecticides against the grain weevil (*Calandra granaria* L.), which readily succumbs to DDT, these analogues showed little or no activity, which seems to invalidate the essential metabolite hypothesis.

The possibility that the efficacy of DDT is dependent upon some intrinsic property of the $-CCl_3$ group has already been hinted at by Erlenmeyer, Bitterli & Sorkin (1948), and the suggestion that the electron-attracting capacity of this group is all-important has been advanced by Skerrett & Woodcock (1950). This is supported by the high activity reported by Hass (1949) for the compounds I and II, and the similarity in the mode of action of these compounds which is apparent from the bioassay method, is further evidence that ready dehydrochlorination is not a prerequisite of insecticidal activity. The reason for the non-toxicity of the nitroethane (III) in our tests is still rather obscure.

The synthesis of further compounds possessing strongly electron-attracting groups on the β-carbon atom has been undertaken in an attempt to substantiate this hypothesis. The quaternary ammonium salts (IV; R = H or CH_3) proved non-toxic to *Calandra granaria*, however, probably because of low lipoid solubility, a property to which the very low toxicity of the trifluoro analogue of DDT (V) has been ascribed by Kirkwood & Phillips (1946).

Further work on the synthesis of compounds possessing suitable electrical status on the β-carbon atom, without loss of lipoid solubility, is in progress.

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Fibrinogen Monolayers at the Air-Water Interface. By D. D. Eley, R. A. Grant and K. B. Taylor. (*Chemistry Department, University of Bristol*)

Earlier workers (Gorter, Maaskant & Van Lockeren Campagne, 1936) found it necessary to pretreat fibrinogen with trypsin in order to form monolayers on a Langmuir trough. We have found, however, that a 0·02 % solution of human fibrinogen (kindly supplied by Dr R. A. Kekwick) in 5 % sodium chloride gives a very satisfactory monolayer on 20 % ammonium sulphate solution. Interaction of this film with thrombin and egg albumin has been followed by the technique of Schulman and Rideal. The method used was the injection of 0·1 ml. of a 0·02 % solution of human thrombin* in 5 % sodium chloride just underneath the fibrinogen or other monolayer held at constant area, the initial surface pressure being 10 dynes/cm. In the case of thrombin under fibrinogen an increase of surface pressure of 10 dynes in 15 min. was recorded. The effect, however, is non-specific, as a similar effect was observed when egg albumin was injected. With other combinations of two proteins a variety of effects was observed. The results obtained were as follows, where the first letter denotes the monolayer and the second the injected protein, and F denotes fibrinogen, E egg albumin, T thrombin and T_b a thrombin solution inactivated by heat:

F/T	F/E	F/T_b	E/T_b	E/T
+	+	±	±	+
T/F	E/F	T_b/F	T_b/E	T/E
-	-	-	+	+

It is suggested that the rise in pressure noted in 10 min. as indicated above is due to adsorption of all or nearly all the injected protein on to the under-

side of the monolayer. We might then expect that the second layer proteins are in their native state, except in the case of the heat-inactivated thrombin, and in this case their effect on the pressure may be supposed to be roughly proportional to the number of molecules present in the given weight injected, and thus inversely as the molecular weight. While we do not know the 'molecular weight' of the thrombin preparation it might be supposed to lie between those of egg albumin and fibrinogen, and the heated thrombin to be somewhat aggregated. The order of increasing molecular weights would then be egg albumin, thrombin, heated thrombin and fibrinogen, which is parallel to the effect on the surface pressure, but obviously more work is necessary before a hypothesis may be safely ventured.

A specific surface clotting action of thrombin on a fibrinogen film, such as might have caused a contraction of the film, was probably ruled out by the high ionic strength of the substrate, apart from other possible factors.

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Kinetic Investigations on Pseudo-Cholinesterase. By D. D. Eley and Gwyneth S. Stone. (*Chemistry Department, University of Bristol*)

Unpublished work in this laboratory has established the close similarity between the non-enzymic hydrolysis of acetylcholine bromide and non-ionic esters such as methyl acetate. Thus Mr G. S. Annis has found the heat of hydrolysis by OH^- ion to be 12,400 cal./g.mol, and hence for hydrolysis by water the heat will be − 1100 cal./g.mol. This shows acetylcholine to be a 'low-energy' compound, as has been assumed by Nachmansohn (1945) in his consideration of the energetics of nerve action. Together with Miss Joan Butterworth we have found the activation energies to be 16,900 cal./g.mol. for the hydrogen-ion catalysed reaction and about 12,000 cal. for the hydroxyl-ion catalysed reaction, values quite close to those found for methyl acetate.

Considering the reaction catalysed by serum cholinesterase (kindly supplied by Dr D. R. Davies), we have investigated the temperature coefficient of the zero order velocity constant which is found at high substrate concentrations. According to the Michaelis theory, this should give the activation energy for the decomposition of the enzyme-substrate complex. In the range 15–45° we find a value of 7900 cal., somewhat larger than the value of 5700 cal. that may be calculated from the results of Glick (1939). However, the value is close enough to values reported for pancreatic lipase, 7600 cal., and grasshopper esterase, 5700 cal. (Sizer, 1943), where we are not concerned with the ionic nitrogen atom, to suggest that the main point of adsorption of the pseudo-esterase is the ester group of the acetylcholine molecule. The activation energy data so far obtained thus confirm the picture of the active groups of this enzyme advanced on more general grounds some time ago by Zeller & Bissegger (1943). It is hoped that further data on activation energies and entropies, together with the corresponding thermodynamic data for the formation of the intermediate complex from the reactants, will help to give a more detailed picture of the activated complex for this reaction than is available at present.

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DEMONSTRATIONS

A Simple Flame Photometer for Routine Estimation of Sodium and Potassium. By G. K. McGowan and J. Smart. (*Department of Pathology, Bristol Royal Hospital, Bristol* 2)

The flame photometer is of the balanced twin photocell type using lithium for the internal standard, as described by Berry, Chappell & Barnes (1946). The most important part is the atomizer, for which a 'Conograph' air-spray brush is used with slight modification. The mist is fed into an air-acetylene flame. The emitted light is conducted by glass rods, through suitable filters, to Megatron 'infrared' barrier layer photocells. The sodium or potassium output is balanced against the lithium output by potentiometer. The apparatus is easy to construct and capable of an accuracy of better than 1 %.

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Berry, J. W., Chappell, D. G. & Barnes, R. B. (1946). *Industr. Engng Chem.* (Anal. ed.), **18**, 19.

The Synthesis of the Pentose Sugars. By L. Hough and J. K. N. Jones. (*Department of Physical and Inorganic Chemistry, University of Bristol*)

Methods of Investigating the Assimilation of Nitrogen by Barley Roots. By E. W. Yemm. (*Department of Botany, University of Bristol*)

The growth of barley roots capable of rapid assimilation of nitrogen will be demonstrated. Methods of determining the effect of N sources on respiration, the analysis of roots and the preparation of N samples for estimation in the mass spectrometer were also shown.

The Microbiological Assay of Amino-Acids in Proteins. By B. F. Folkes and E. W. Yemm. (*Department of Botany, University of Bristol*)

The method is a modification of that of Henderson & Snell (1948), utilizing $10 \times 2{\cdot}5$ cm. flat-bottomed specimen tubes. Growth is estimated after 3 days' incubation at 37° by titration of the lactic acid produced to pH 7·3 using the glass electrode.

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The Analysis of Inorganic Compounds by Paper Chromatography. By F. H. Pollard, J. F. W. McOmie and H. M. Stevens (introduced by C. A. Ashford). (*Department of Chemistry, University of Bristol*)

Inorganic chromatography on filter paper and cellulose columns has provided a new technique with many potential uses. The methods and apparatus used for the separation of inorganic cations and anions, and their detection by ultraviolet fluorescence and other methods were demonstrated.

An Interferometer Suitable for Electrophoresis. By C. R. Burch and A. F. Rogers (introduced by C. A. Ashford). (*Departments of Physics and Physiology, University of Bristol*)

Light is divided at a half-silvered surface, sent both ways round a rhomboid, and re-united at a second half-silvered surface at the opposite corner, so as to give white light interference fringes. This type of 'round the square' interferometer is well known to opticians. It was originally described by Mach-Zehnder many years ago, and has been applied—chiefly in Germany—to the study of air flow in wind tunnels.

If a fluid cell is placed in each arm, the interference fringes show the transit-time (refractive index × thickness) difference between the two arms. With a suitable adjustment the fringes themselves may be made to be graphs of refractive index in a horizontally stratified heterogeneous liquid, such as exists in an electrophoresis or diffusion cell. With a cell 1 cm. thick a displacement of one interference fringe is produced by a change of refractive index of 0·00005. Changes of one-tenth of a fringe are detectable.

The fringes may be observed directly or projected on to a screen or photographed. They are localized in the superposed fluid cell so that each fringe constitutes a graph of refractive index distribution, integrated along the line of sight occupied by it in the cell but not also averaged transversely. This may be contrasted with fringes obtained in interferometers of the second class in which the cells to be compared lie in different parts of the same (coherent) wave front, instead of in a wave front and its *alter ego*. In the second class of interferometer the fringes, of necessity, cannot have horizontal as well as vertical localization in the cell, and though they may be made to constitute graphs of vertical refractive index distribution, it is the horizontal average across as well as along the line of sight that is so graphed. Neither method of representation can be termed intrinsically right, it is simply a question of which quantity we wish to observe—strictly local or average refractive index.

The two types of instrument may also be contrasted in that the coherence of wave front (necessary in instruments of the second class) implies a light source of small angular width and hence a relatively restricted amount of light. This restriction is avoided in the present instrument by the creation of an *alter ego* wave front at a half-silvered surface, and enough light can thus be obtained to permit projection.

The Schultz Reaction for Cholesterol and Cholesterol Esters in the Rat Suprarenal. By C. A. Ashford, Frances B. Robinson and J. M. Yoffey. (*Departments of Physiology and Anatomy, University of Bristol*)

Apparatus for Following Hydrogen-Exchange Reactions Catalysed by Bacterial Suspensions. By A. Couper (introduced by D. D. Eley). (*Chemistry Department, University of Bristol*)

The exchange of atoms between molecular deuterium and water catalysed by bacterial suspensions was first investigated by Polanyi, Cavanagh & Horiuti (1934), and by Farkas, Farkas & Yudkin (1934). An apparatus is demonstrated for the investigation of this reaction, together with the rate of parahydrogen conversion. Provision is made for shaking the bacterial suspension with hydrogen gas, and its subsequent removal for analysis by a measurement of thermal conductivity in a micropirani gauge, according to the method of Bolland & Melville (1937). A certain economy of resources has been effected by introducing simplifications into the electrical circuit. The apparatus is being used to investigate the behaviour of *Escherichia coli* and *Proteus vulgaris* (kindly provided by Prof. K. E. Cooper).

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **297th Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University of Oxford, South Parks Road, Oxford, on Saturday, 5 May* 1951, *when the following papers were read:*

COMMUNICATIONS

The Separation of Polyphenols in Tea Leaf by Paper Chromatography with Water as a Mobile Solvent.

By E. A. H. Roberts and D. J. Wood. (*Tocklai Experimental Station, Indian Tea Association, Cinnamara, Assam, India*)

It has been found that water is a useful solvent for paper chromatography of the polyphenols occurring in tea leaf. In combination with *n*-butanol-acetic acid two-way chromatograms are obtained which give satisfactory separations of individual polyphenols.

The chromatograms obtained with fresh tea-leaf juice show spots provisionally identified with the following catechins: (−)-*epi*gallocatechin, (+)-gallocatechin, (−)-*epi*catechin, (+)-catechin, (−)-*epi*gallocatechin gallate and (−)-*epi*catechin gallate. In addition, the chromatograms show spots corresponding with gallic acid, *m*-digallic acid (?), chlorogenic acid, rutin, quercitrin, and a considerable number of trace substances, so far unidentified, which show up on the chromatograms either after exposure to ammonia vapour or after spraying with ferric alum or ammoniacal silver nitrate. Amino-acids and sugars run nearly as far as the solvent front with water.

If the juice is boiled or autoclaved before chromatography, fresh spots appear on the chromatogram, presumably as a result of the following epimeric changes (Freudenberg, 1933):

(−)-*epi*gallocatechin	→ (−)-gallocatechin
(+)-gallocatechin	→ (+)-*epi*gallocatechin
(−)-*epi*catechin	→ (−)-catechin
(+)-catechin	→ (+)-*epi*catechin
(−)-*epi*gallocatechin gallate	→ (−)-gallocatechin gallate
(−)-*epi*catechin gallate	→ (−)-catechin gallate

The separation on the chromatogram of pairs of optical isomers such as (+)- and (−)-gallocatechin therefore appears likely, so that paper chromatography, with water as solvent, has apparently resolved mixtures of optically active isomers. As the separations with water are presumably due to adsorption and not partition effects, such a separation is theoretically quite possible, as the cellulose may be considered an optically active adsorbent.

The separations are only appreciable with water as solvent, and not with *n*-butanol-acetic acid.

With flavonols, adsorption of aglycones by filter paper is very strong, quercitin and myricetin having R_F = zero with water as solvent. The glycosides of these flavonols are less strongly adsorbed (R_F = 0·3–0·4). This difference may be used as the basis of a test to distinguish between these aglycones and their glycosides.

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Isolation of Unequivocal Uroporphyrin III. A further study of Turacin.

By R. E. H. Nicholas and C. Rimington. (*Department of Chemical Pathology, University College Hospital Medical School, London, W.C.* 1)

Two isomeric series of porphyrins are encountered in nature, the I series derivable from aetioporphyrin I (1:3:5:7-tetramethyl-2:4:6:8-tetraethyl porphin) and the III series derivable from aetioporphyrin III, in which the 7 and 8 substituents are interchanged. All haemoproteins of physiological importance, such as haemoglobin, catalase, cytochrome *c*, etc., belong to the III series; nevertheless, coproporphyrin I does occur naturally and is the chief porphyrin of normal excreta. It is generally regarded as a by-product of haemoglobin biosynthesis (Rimington, 1938).

In porphyria, there is a greatly increased excretion of coproporphyrin, mainly series I in the congenital disease and mainly series III in the acute disease; the structure of both these porphyrins has been established by synthesis. Urinary uroporphyrin I excretion was first found by Fischer (1915) in his

study of the congenital porphyria patient Petry. The structure porphin-1:3:5:7-tetraacetic-acid-2:4:6:8-tetrapropionic acid was assigned to uroporphyrin I on the grounds of its partial decarboxylation to coproporphyrin I and the elimination of other possible structures, disproved by synthesis (Fischer & Hofmann, 1937; Fischer & Zischler, 1937).

The urine of acute porphyria patients was shown by Waldenström (1936) and Mertens (1936, 1937) to be rich in a uroporphyrin which, because of its decarboxylation to coproporphyrin III, they regarded as uroporphyrin III. This view was accepted until Grinstein, Schwartz & Watson (1945) and Watson, Schwartz & Hawkinson (1945) claimed to have resolved chromatographically such material from acute porphyria cases into uroporphyrin I and a heptacarboxylic porphyrin of the III series. Unequivocal uroporphyrin III has not yet been obtained by any investigator.

Modern work upon the biosynthesis of haem makes it very probable that the first porphyrin to be synthesized is uroporphyrin, belonging of necessity to the III series, since the protoporphyrin which is postulated to be derived from it is a series III pigment. The isolation and characterization of uroporphyrin III thus became a matter of first importance.

Turacin, the red-feather pigment of certain African birds, was stated by Fischer & Hilger (1923, 1924) to be uroporphyrin I copper complex, but Rimington (1939) obtained only coproporphyrin III by decarboxylation of turacin porphyrin. Hence it seemed probable that turacin would prove to be a good source of the desired uroporphyrin III. This expectation has been fulfilled.

The crude porphyrin (450 mg.) from turacin has been purified by chromatography and two main fractions obtained. The larger was an eight-carboxyl porphyrin, the octamethyl ester of which crystallized in hair-like needles, m.p. 264°. (Found: C, 61·9; H, 6·4; N, 5·8; MeO, 26·5. Uroporphyrin III octamethyl ester ($C_{48}H_{54}O_{16}N_4$) requires C, 61·1; H, 5·8; N, 5·9; MeO, 26·3%.) The coproporphyrin ester obtained by partial decarboxylation yielded crystals, m.p. 153°, remelt 179°, unchanged by recrystallization, and by concentration of the first mother liquors a further crop also melting at 153°, remelt 179°, was obtained. Only the III series coproporphyrin ester was therefore present, indicating that the parent uroporphyrin was also uroporphyrin III alone.

The second porphyrin fraction isolated by chromatography (about 10% of the total) was also an eight-carboxyl porphyrin ester, m.p. 210°. Attempted decarboxylation by the usual method led only to colourless products. Micro-analysis suggests a porphyrin with four CH_2 groups less than uroporphyrin, e.g. a porphin octaacetic acid.

In addition to these porphyrins, two materials were isolated, in traces only, having chlorin and phyllo type spectra respectively. They are regarded as chemical artifacts.

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The Hexokinase Activities of Rat Tissues. By C. Long. (*Department of Biological Chemistry, Marischal College, Aberdeen*)

Hexokinase activity has been measured in homogenates of rat tissues by determining the rate of disappearance of glucose from a medium of the following composition (final concns. in brackets): glucose (0·0012–0·0024 M), adenosinetriphosphate, (ATP) (0·005M), $MgCl_2$ (0·005M), KF (0·05M), potassium phosphate buffer, pH 7·8 (0·04M), KCl (0·042M). The total volume was 0·50 ml. and included 0·20 ml. of a 1 in 6 homogenate of tissue (equivalent to 33 mg. moist wt.). Incubation was at 30° in open tubes. Glucose was determined according to Nelson (1944). The concentrations of substrates and cofactors were optimal for those tissues studied, and the rate of reaction varied linearly with the volume of homogenate used; for most tissues the rate of glucose phosphorylation was strictly proportional to duration of incubation, which was generally not longer than 5–10 min.

The hexokinase activities have been expressed as $-Q_{glucose}$ (μl. glucose utilized/mg. dry wt. of tissue/hr., where 180 μg. glucose = 22·4 μl.), and the following average values have been obtained: brain, 27·1; stomach, 17·7; colon, 17·2; heart, 14·9; testis, 14·0; small intestine, 11·7; caecum, 10·9; uterus, 9·3; kidney, 7·5; skeletal muscle, 6·0; spleen, 6·0; pancreas, 5·9; lung, 4·3; liver, 1·4. Where comparisons with data given in the literature are available, it appears that homogenates have a higher hexokinase activity than extracts.

In agreement with previous workers (Reiner, 1947; Utter, 1950), the rate of glucose phosphorylation in brain is about twelve times the known rate of respiration. The low hexokinase activity of liver, also found by Vestling, Mylroie, Irish & Grant (1950), when taken in conjunction with other evidence, suggests that the scheme

glucose → glucose-6-phosphate → glucose-1-phosphate → glycogen

is not a major pathway in liver. The hexokinase activities of intestine and kidney, as determined in this work, are too low to account for the phosphorylation of those amounts of glucose known to be absorbed from the gut and glomerular filtrate, respectively.

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The Reactivity of the Labile Phosphate Groups of Adenosinetriphosphate. By H. A. Krebs, Monica Johnson, L. V. Eggleston and R. Hems. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, The University, Sheffield* 10)

Adenosinetriphosphate (ATP) is known to undergo rapid dephosphorylation and resynthesis in metabolically active tissues, but it is still uncertain to what extent ATP is broken down to, and resynthesized from, adenosinediphosphate (ADP), according to scheme 1, or adenosinemonophosphate (AMP), according to scheme 2:

Scheme 1.

Scheme 2.

The following experiments show that both types of cycle occur, scheme 1 being the main mechanism in respiring muscle and scheme 2 in respiring liver.

Tissue homogenates were incubated aerobically at 20° in the presence of about 0·002 M-ATP and 0·004 M-inorganic orthophosphate. At the beginning of the incubation period $^{32}PO_4$ was added, and at intervals the specific activities of ATP and inorganic orthophosphate were measured. The phosphate fractions were separated by paper chromatography according to the methods of Hanes & Isherwood (1949). The spots were detected by means of the molybdate reaction, cut out and used for quantitative phosphate determinations and for the measurement of the radioactivity. In pigeon-breast muscle homogenates the specific activity of ATP rapidly rose, and that of inorganic phosphate rapidly fell, until after 10–20 min. the specific activity of ATP-phosphorus was about 33 % of that of inorganic phosphorus. Thereafter there was a very slow further increase in the specific activity of ATP and a fall in that of inorganic phosphate, but the rate of this change was only 1–2 % of that of the initial reaction. In respiring muscle, therefore, the terminal phosphate group of ATP reacts 50–100 times more rapidly than the second phosphate group. In similar experiments on rat-liver homogenates a stationary state was reached within 20–30 min., when the specific activity of ATP was about 67 % of that of inorganic phosphate (see Table 1). In respiring liver, therefore, the reactivity of both labile phosphate groups of ATP is of the same order.

Table 1. *Changes in the specific activity of ATP and PO_4 on addition of $^{32}PO_4$ to tissue homogenates*

(Medium: 60·7 ml. saline III of Krebs (1950) omitting organic compounds, $CaCl_2$ and phosphate; 2·8 ml. 0·1 M-phosphate buffer, pH 7·4; 1 ml. 0·1 M-$MgCl_2$; 0·002 M-ATP; 0·01 M-succinate. 20°. Tissue concentration: 3·3 % liver; 5 % pigeon-breast muscle.)

Tissue	Ratio: Specific activity of ATP phosphorus / Specific activity of inorganic phosphorus after 10 min.	20 min.	30 min.	60 min.
Rat liver	0·46	0·58	0·67	0·68
Pigeon-breast muscle	0·29	0·35	0·37	0·40

Zetterström, Ernster & Lindberg (1950) have reported that the stable phosphate group of ATP is more readily renewed in liver than in muscle. This may be connected with the present observation that the renewal rate of the second labile phosphate group is much greater in liver than in muscle.

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The Uptake of ^{32}P by Ribonucleotides in Liver-Cell Fractions. By J. N. Davidson, W. M. McIndoe and R. M. S. Smellie. (*Department of Biochemistry, University of Glasgow*)

The method for the separation of ribonucleotides by ionophoresis from the ribonucleic acid (RNA) fraction in the Schmidt & Thannhauser separation scheme, recently described by Smellie & Davidson (1951), has been applied to isolated nuclei and cytoplasmic fractions from the liver tissue of adult rats which had received 20 μc./100 g. of ^{32}P 2 hr. prior to killing.

Jeener (1949) for mouse embryo, chick embryo and pigeon crop gland, although Jeener & Szafarz (1950) found in resting liver a higher activity in small granules than in large granules. Barnum & Huseby (1950) have recorded higher activities in cell sap than in small granules in liver.

We have found that, in general, uridylic acid in the cytoplasmic RNA fractions shows a higher

Component of ribonucleotide fraction	Specific activities (counts/min./100 μg. P) Whole tissue	Isolated nuclei	Large granules	Small granules	Cell sap
Cytidylic acid	300	2900	204	65	375
Adenylic acid	285	3300	216	128	260
Guanylic acid	121	1925	188	33	135
Uridylic acid	482	2660	325	120	314
Inorganic P (from 'phosphoprotein')	3050	6020	3600	4000	4660
Organic P	660	—	500	50	180
Whole fraction	825	2770	785	530	725

The results show that the activity of the nuclear RNA is much greater than that of the RNA in any cytoplasmic fraction. This is in agreement with the findings of Marshak (1948), Marshak & Calvet (1949), Barnum & Huseby (1950), and Jeener & Szafarz (1950).

Of the cytoplasmic RNA fractions the activity is lower in the small granules than in the large granules or cell sap. Similar results are recorded by

activity than do the other nucleotides, whereas in nuclear RNA adenylic acid shows the greatest activity.

The 'phosphoprotein' phosphorus in all fractions showed a very high activity. In the cytoplasmic fractions it was accompanied by small amounts of a non-nucleotide organic phosphate fraction which appeared to consist in part of ethanolamine phosphate.

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Pancreatic Trypsin Inhibitor. By N. M. GREEN and ELIZABETH WORK. (*Department of Chemical Pathology, University College Hospital Medical School, London, W.C.* 1)

The pancreatic trypsin inhibitor crystallized by Kunitz & Northrop (1936) was isolated from commercial cattle pancreas residues after insulin extraction. Preparations containing 1–2% of inhibitor were obtained by deproteinizing the 0·8 saturated ammonium sulphate precipitate from acid extracts of pancreas residues. After sodium chloride fractionation between pH 5·5 and 3 the material was electrodialysed. The inhibitor migrated through cellophan to the cathode, when the pH of the centre was below 10. The catholyte, containing 50% pure inhibitor, was further purified through the crystalline compound with trypsin (Kunitz & Northrop, 1936). Overall yield of recrystallized inhibitor was about 1 mg./kg. pancreas residues.

The specific property solubility test (Falconer & Taylor, 1946), using potassium phosphate buffer at pH 6·5, gave no evidence of impurities in the inhibitor, the activity concentration ratios in the liquid phase being constant.

The properties of the inhibitor are those of a basic peptide, although minimum solubility is exhibited at pH 3 in water or salt solutions. It is stable in neutral or acid solution, but is slowly inactivated by alkali. The molecular weight (based on the osmotic pressure, kindly determined by Dr H. Gutfreund) is 9000 ± 500. The approximate amino-acid composition, determined mostly by paper chromatography (Dent, 1948; Work, 1949), is shown in Table 1. Histidine and tryptophan are the only common amino-acids not present. An unidentified spot in the position of methionine sulphoxide was found in chromatograms from peroxide-treated hydrolysates. End-group assay by the fluorodinitrobenzene method of Sanger (1945) showed arginine as the terminal amino-acid in amounts not greater than one end-group/molecule of inhibitor.

Inhibitor and trypsin activities were determined by modification of the method of Anson (1938), using denatured crystalline carboxyhaemoglobin as substrate. One g.mol. of inhibitor inactivated 0·9 g.mol. of trypsin, suggesting equimolecular combination. Identical combining ratios were found with soya-bean inhibitor (gift from Dr M. Kunitz).

Table 1. *Amino-acids in trypsin inhibitor, estimated by paper chromatography (error of estimation, ± 15% of quantity measured)*

Amino-acid	% by weight	Molecules/molecule inhibitor
Aspartic acid	8	6
Glutamic acid	8	6
Cystine	2·5	1
Threonine	4·5	4
Serine	3·5	3
Glycine	7·5	10
Alanine	14·5	18
Valine	2·5	2
Proline	5·5	5
Leucine and isoleucine	6	5
Phenylalanine	8·0	5
Tyrosine*	9·0	5
Tryptophan*	0	0
Methionine	1·5	1
Lysine†	7·3	5
Arginine	15	9
Histidine	0	0

* Determined spectrophotometrically (Goodwin & Morton, 1946).

† Determined through its dinitrophenyl derivative.

The inhibition was of a non-competitive type, being unaffected by fourfold variation of substrate concentration (cf. ovomucoid inhibitor, Fraenkel-Conrat, Bean & Lineweaver, 1948). The findings of Kunitz & Northrop (1936) that the inhibitor-trypsin combination is not instantaneous and is reversed at pH 3 were confirmed.

We are most grateful to British Drug Houses Ltd. for providing the pancreas residues and for carrying out the preliminary extractions.

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The Metabolism of Aliphatic Alcohols. Glucuronide Formation. By I. A. KAMIL, J. N. SMITH and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*)

Alcohols are apparently dealt with in the body in three ways, namely, (*a*) elimination in the unchanged state, (*b*) oxidation and (*c*) conjugation. The extent to which an alcohol is metabolized along any of these paths appears to depend largely upon the extent of branching of the hydrocarbon chain. Other factors are also involved. Haggard, Miller & Greenberg (1945) showed that for the amyl alcohols the rate of oxidation is primary > secondary > tertiary. Our present preliminary studies indicate that for conjugation with glucuronic acid the sequence is, in general, tertiary > secondary > primary, although some secondary alcohols are very highly conjugated (see Table 1). This suggests that that part of the alcohol which is not oxidized is conjugated.

Table 1. *The glucuronic acid conjugation of some aliphatic alcohols*

Alcohol	Dose (in mmol./rabbit)	Glucuronide (% of dose)
Primary		
(*a*) Normal:		
CH_3OH	75	0
C_2H_5OH	50	0
$CH_3OH + C_2H_5OH$	125	1
C_3H_7OH	40	0·9
C_4H_9OH	16	0
$C_7H_{15}OH$	25	5
$C_8H_{17}OH$	25	9
(*b*) Arborescent:		
$(CH_3)_2CHCH_2OH$	25	4·4
$CH_3.C_2H_5CHCH_2OH$	25	7
$(C_2H_5)_2CHCH_2OH$	25	40
Secondary		
(*a*) Straight chain 2-ols:		
$CH_3CHOHCH_3$	50	10
$C_2H_5.CHOHCH_3$	25	14
$n\text{-}C_6H_{13}.CHOHCH_3$	25	15
(*b*) Branched chain 2-ols:		
$iso C_4H_9.CHOHCH_3$	25	35
(*c*) 4-ols:		
$n\text{-}C_3H_7.CHOH.n\text{-}C_3H_7$	25	68
Tertiary		
$(CH_3)_2C.OH$	12	25
$(CH_3)_2.(n\text{-}C_3H_7).C.OH$	25	57

Briefly our results suggest the following comments.

Primary alcohols. (*a*) *Normal.* Conjugation is small, but increases as the series is ascended. These alcohols are rapidly oxidized.

(*b*) *Arborescent.* The two containing β-methyl groups (β:β-dimethylethanol and β-ethyl-β-methylethanol) form about 5% glucuronide, and are presumably extensively destroyed *in vivo*. The two containing β-ethyl groups (β:β-diethylethanol and β-*n*-butyl-β-ethylethanol) give rise to highly reducing urines containing large amounts of ester glucuronide, probably of the corresponding acids, diethylacetic acid and *n*-butylethylacetic acid. Dziewiatkowski, Venkataraman & Lewis (1949) have already shown that diethylacetic acid gives rise to an ester glucuronide. This acid was isolated in large amounts from diethylethanol urine. Methyl *n*-propyl ketone was also isolated indicating the following route of metabolism for diethylethanol:

$$CH_3CH_2CH(C_2H_5)CH_2OH \longrightarrow CH_3CH_2CH(C_2H_5)COOH \longrightarrow CH_3COCH(C_2H_5)COOH \xrightarrow{-CO_2} CH_3COCH_2C_2H_5.$$

Secondary alcohols. Preliminary results suggest that there may be, from a metabolic aspect, several groups.

(*a*) *Straight chain 2-ols.* These are mainly oxidized via ketones (proved by isolation), there being also some 10–15% glucuronide formation.

(*b*) *Branched chain 2-ols.* Our one example suggests high glucuronide formation due to branching of the chain.

(*c*) *4-ols.* Here, one example suggests exceptionally high glucuronic acid conjugation. *Di-n-propylmethylglucuronide* was isolated (*triacetyl methyl ester*, m.p. 103°).

Tertiary alcohols. Tertiary butyl and tertiary amyl alcohols are known to form glucuronides (Thierfelder & Mering, 1885). These alcohols are probably slowly oxidized *in vivo* (Haggard *et al.* 1945), and our measurements on two of these show high conjugation in keeping with extreme branching of the hydrocarbon chain. The glucuronide of 1:1-dimethylbutylcarbinol was isolated (*triacetyl methyl ester*, m.p. 104°).

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The Nucleic Acid of Egg White. By H. FRAENKEL-CONRAT and E. D. DUCAY. (*Western Regional Research Laboratory, U.S. Department of Agriculture, Albany, California*)

It has recently been shown that avidin, as usually isolated, is a nucleoprotein (avidin NA) (Fraenkel-Conrat, Ducay, Snell & Ward, 1950). The water-soluble protein moiety (avidin A) alone, however, carried the specific biotin binding activity.

Nucleic acid appears not to have been previously demonstrated in, or isolated from, egg white. The finding that the nucleic acid was of the deoxypentose type was particularly surprising. This has since been further established by its digestibility with deoxyribonuclease, and its resistance to ribonuclease. Quantitative analyses of the constituent bases indicate a composition resembling most deoxypentose nucleic acids (for 4 units : 1·19, 0·86, 0·85 and 1·10 for adenine, guanine, cytosine and thymine, respectively) (Markham & Smith, 1949).

The question arose whether the isolation of the nucleic acid in complex with avidin represented its true state in egg white or whether the formation of this complex occurred during isolation. Experiments in which deoxypentose nucleic acid labelled with ^{32}P was added to egg white prior to isolation of the avidin NA indicated that only a small fraction of the nucleic acid associated with avidin was labelled, which supports the belief that the complex occurs as such in egg white, and is relatively stable. Another finding favouring this concept is that while the total nucleic acid and avidin A content of egg white is comparatively constant, the amount of avidin NA that can be isolated by the same technique varies greatly for different egg white preparations.

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The Distribution of Vitamin A in Male and Female Rats at Different Levels of Dosing. By T. MOORE, I. M. SHARMAN and R. J. WARD. (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*)

The investigation recently reported by Moore & Sharman (1950) has been extended by giving numerous additional groups of rats of each sex daily doses of 160–10,240 i.u. of vitamin A acetate. As before, the rats were kept on a diet deficient in vitamin A for a period calculated to exhaust their reserves, and were then dosed for 4–6 weeks until 2 days before killing. Vitamin A was estimated by the $SbCl_3$ method in the livers and kidneys of individual animals, and in the pooled blood, lungs, adrenals and intraperitoneal fat of groups. The results obtained in this investigation for the livers, kidneys and blood are combined with those obtained in the earlier work in Table 1.

In confirmation of the work of Lemley, Brown, Bird & Emmett (1947) and Davies & Moore (1948) it was found that the percentage efficiency of storage of vitamin A in the combined liver and kidneys at first increased with the size of the dose, then reached a maximum and finally decreased when the doses verged on the toxic level. During the first phase storage was rather more efficient in females than in

Table 1. *Average concentrations of vitamin A in male and female rats at different levels of dosing*

Average time dosed (days)	Daily dose vit. A (i.u.)	No. of rats		Liver (i.u./g.)		Kidneys (i.u./g.)		Blood (i.u./ml.)		% stored	
		M	F	M	F	M	F	M	F	M	F
36	10	3	3	0·4	0·4	2·0	2·3	0·37	0·31	3	2
35	20	3	3	0·6	5·2	8·8	8·1	0·56	0·57	3	6
32	40	12	7	10·4	24·4	37·5	4·9	0·94	0·79	15	13
29	80	8	8	39	98	15·4	3·7	1·06	0·60	17	26
34	160	3	3	80	153	11·7	4·6	0·90	0·64	15	18
37	320	11	6	435	755	8·2	4·7	0·98	0·76	39	42
38	640	4	4	1,710	2,680	10·3	21·0	0·90	0·77	80	78
41	1,280	4	4	3,250	4,300	18·0	8·2	1·75	1·50	59	66
42	2,560	3	3	3,290	6,960	8·7	8·7	3·27	1·15	31	45
43	5,120	3	3	8,380	12,200	37·9	29·5	0·85	0·98	36	36
44	10,240	3	2	14,500	16,300	228	112	1·44	0·99	31	26

males, but at high doses this difference was usually eliminated. Between dosages of 40 and 320 i.u. the concentrations of vitamin A in the kidneys were much greater in males than in females, but concentrations in the liver were greater in females than in males. Over the same range of dosing, moreover, a surprising decline in the concentration of vitamin A in the male kidneys was observed as the dose was increased. Thus with a dose of 40 i.u. the kidneys averaged 37·5 i.u./g., but with 320 i.u. only 8·2 i.u./g. In parallel experiments two groups of male rats given these two levels of dosing grew at almost identical rates. In the females the same effect, if present, was obviously on a much smaller scale, with averages of 8·1 i.u. at a dose of 20 i.u. and 3·7 i.u. at a dose of 80 i.u. With very massive doses, however, high concentrations were again observed, in confirmation of the findings of Davies & Moore (1934), in the kidneys of both sexes. These results suggest that a threshold concentration of vitamin A is necessary in the liver before it can absorb vitamin A more efficiently than the kidneys, and that to reach this threshold higher doses must be given to males than to females.

The results for other tissues, which were pooled, were based on fewer analyses. The concentrations of vitamin A in the blood tended to be higher in males than in females. In both sexes, however, no consistent relation could be found either with the dose or the liver reserves, except perhaps that low values were found at low levels of dosing. With massive doses, in confirmation of the work of Davies & Moore (1934), high concentrations of vitamin appeared in the lungs and adrenals in both sexes, with values of up to 430 and 380 i.u./g., respectively. The intraperitoneal fat contained only traces (2–3 i.u./g.) at doses of under 320 i.u., but reached about 100 i.u. at the maximum dose given. Under ultraviolet irradiation yellow fluorescence was observed in the fat deposits after doses of 160 i.u. or more and in the isolated fat after 2560 i.u.

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Some Steroids of Pregnant Mares' Urine. Separation by Chromatography of Benzoates. By R. V. Brooks, W. Klyne and E. Miller. (*Postgraduate Medical School, London, W.* 12)

Conjugated steroids were obtained from pregnant mares' urine by extraction with butanol (Klyne, Schachter & Marrian, 1948) and hydrolysed with acid. The free non-phenolic, non-ketonic steroids obtained by the usual separations were fractionated with digitonin following Marker, Rohrmann & Wittle (1938), and the fraction precipitated in 95% (v/v) ethanol (β1 fraction) has been examined in detail. Chromatography of the steroid alcohols yielded some 'uranediol' (17-methyl-D-homoandrostane-3β:17a-diol; Marker, Rohrmann & Wittle, 1938; Klyne, 1948, 1950) and *allo*pregnane-3β:20β-diol (Bauld & Heard, 1940). The intermediate fractions and mother liquors from the separation of these compounds were benzoylated. Chromatography of the benzoates on alumina yielded very sharp separations; light petroleum (b.p. 60–80°)-benzene mixtures of increasing benzene content eluted in turn the dibenzoates of *allo*pregnane-3β:20β-diol (predominant in quantity), *allo*pregnane-3β:20α-diol (cf. Marker & Rohrmann, 1938), compound *M* and uranediol. Compound *M* dibenzoate had double m.p. 163–164° and 191–192° (corr.); $[\alpha]_D^{20°} - 15°$ in $CHCl_3$. (Found: C, 79·7; H, 8·1. $C_{35}H_{44}O_4$ requires C, 79·5; H, 8·4%.) Compound *M* had m.p. 184–185° (corr.), $[\alpha]_D^{20°} - 6°$ in ethanol; its acetate had m.p. 108–109° (corr.), $[\alpha]_D^{20°} - 14°$ in $CHCl_3$.

This appears to be the first reported isolation of a pair of 20-epimeric steroids from any urine.

The following dibenzoates have been prepared; m.p.'s are corrected; figures in square brackets are specific rotations in $CHCl_3$ at 20–25°: *allo*pregnane-3β:20α-diol, m.p. 171–172°, [+27°]; pregn-5-ene-3β:20α-diol, m.p. 194–196°, [+5°]; pregn-5-ene-3β:20β-diol, m.p. 228–230°, [−48°]; uranediol, m.p. 211–213°, [+22°].

Epimeric 20α- and 20β-hydroxy steroids can be distinguished very easily by the molecular rotations of their benzoates. The molecular rotation contributions of the benzoxy groups are approximately as follows: 20α, +110°; 20β, −120°.

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Cholesterol Metabolism in Man. By R. P. Cook and D. C. Edwards. (*Department of Biochemistry, University College, Dundee*)

Cholesterol (10 g.) in olive oil (50 g.) was administered to a human subject (71 kg.). Before administration and 1, 2 and 3 days after, the faeces were collected and the ether- and ethanol-soluble lipids fractionated.

The absorption of cholesterol was approx. 20% of the amount given. As with rats, there is an increased excretion of faecal acids (Cook, Polgar & Thomson, 1950). No marked effect on the level of plasma cholesterol was observed.

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Human Faecal Lipids. By D. C. Edwards and R. P. Cook. (*Department of Biochemistry, University College, Dundee*)

The ether and ethanol extracts of dried human faeces of a subject on a normal diet have been fractionated. Unsaponifiable matter forms 34% of the lipid and total sterol 10%, of which 88% is present in the free form. The sterols have been characterized.

The fatty acids have been separated into volatile and non-volatile acids (Friedmann, 1938), and the volatile acids shown to consist of acetic, propionic, butyric, valeric and hexanoic and higher acids, using the method of Moyle, Baldwin & Scarisbrick (1948).

The non-volatile acids (37% of total lipid) contain 25% light petroleum (b.p. 40–60°)-insoluble acids, and the light petroleum-soluble acids contain 47% solid acids and 29% liquid acids by the Twitchell separation.

Fractional distillation of the methyl esters of the solid acids (Hilditch, 1940) and chromatography (Howard & Martin, 1950) indicate the presence of myristic, palmitic, stearic and higher acids. Oleic acid is the main component of the liquid acid fraction.

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The Effect of Insulin on Cellular Composition and Growth of Chick-Heart Explants. By I. Leslie and J. N. Davidson. (*Biochemistry Department, University of Glasgow*)

Insulin in concentrations of 2–3 units/ml. in a fully adequate growth-promoting medium has been shown to promote cell proliferation in chick-heart explants. The chemical changes associated with this action have been investigated by the technique previously described for the determination of cell number and composition in roller tube cultures (Davidson & Leslie, 1950*a*, *b*, 1951).

The results shown in Table 1 can be summarized as follows:

(*a*) Cell number was significantly greater in the insulin-treated explants at 144 hr.

(*b*) The total amounts of lipid phosphorus (LP), ribonucleic acid phosphorus (RNAP), and protein nitrogen (PN) per 48 explants were increased by insulin.

Table 1. *The effect of insulin on cellular composition and growth of chick-heart explants*

Age (hr.)	Explants	μg. P or N/48 explants					Cell no. × 10^6	μg. × 10^{-7} P or N/cell			
		ASP	LP	RNAP	DNAP	PN		ASP	LP	RNAP	PN
0	Initial	2·7	4·4	1·64	0·58	66	2·5	11·1	17·8	6·63	270
144	Control	37·0	6·8	5·48	1·88	186	8·0	46·3	8·5	6·85	232
144	Insulin	21·7	10·8	10·34	2·78	263	11·8	18·3	9·1	8·75	222
	't' test values for differences between control and insulin groups*	1·52	4·39	6·84	3·03	3·37	3·03	3·12	1·14	2·46	—

* For the group of 12 observations the 't' value corresponding to $P=0·05$ is 2·228. Values greater than this may be taken as statistically significant.

(*c*) Calculating on the basis of a constant amount of $2·35 \times 10^{-7}$ μg. deoxyribonucleic acid phosphorus per cell, it was found that the mean amount of acid-soluble phosphorus (ASP) per cell was decreased, but the RNAP per cell was increased by the action of insulin over a period of 144 hr. There was no significant difference between the amounts per cell of PN and LP in the control and insulin-treated explants.

The evidence that insulin stimulates protein synthesis in cells growing *in vitro* is in direct support of the *in vivo* observations (e.g. Mirsky, 1939) indicating that insulin is a protein anabolic agent. Latta & Bucholz (1939) obtained increased proliferation of chick-heart fibroblasts under the influence of insulin, but only at the abnormally high concentration of 33 units/ml. However, the increased cell proliferation combined with a selective rise in RNAP per cell, which we have obtained with 2–3 units/ml. of insulin, strongly indicates a true hormonal action. It is pertinent to note that insulin increases the ribonucleotide concentration in motor nerve cells (Hyden, 1947), stimulates phosphocreatine synthesis in brain homogenates and promotes ^{32}P uptake by ATP and phosphocreatine in muscle (cf. Goranson & Erulkar, 1949).

The possible influence of other hormones in the growth of chick-heart explants is at present under investigation.

We should like to thank Dr E. Reid, School of Biochemistry, Cambridge, for his co-operation in this work, and Dr J. R. F. Williams, of Boots Pure Drug Co., for supplying us with a sterile solution of insulin free from preservative.

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Observations on Fibrinolysis. Is the Activity Produced by Exercise due to Plasmin? By Ethel Bidwell and R. G. Macfarlane. (*Department of Pathology, Radcliffe Infirmary, University of Oxford*)

Macfarlane & Pilling (1946) and Biggs, Macfarlane & Pilling (1947) have shown that human plasma can become fibrinolytic following strenuous exercise or injection of adrenaline. It appeared that their observations could be explained by assuming an increase in plasmin and a decrease in antiplasmin. We have now shown that: (1) fibrinolytic plasma is just as inhibitory to partially purified plasmin as is normal plasma; (2) the fibrinogen in fibrinolytic plasma does not appear to be attacked at all; digestion does not begin until after the formation of the clot (see also Fantl & Simon, 1948); (3) digestion of the clot as measured by determining quantitatively the amount of clot remaining after a given period of incubation does not begin immediately after the formation of the clot; there may be a long period during which there is no decrease in the amount of clot; (4) clots formed in the presence of calcium ions lyse more slowly than those formed in the absence of calcium ions; (5) mixtures of fibrinolytic and non-fibrinolytic plasmas are actively fibrinolytic to the extent that would be expected if the active agent

were being diluted out without being influenced by an inhibitor.

We have imitated the observation (1) using an 'artificial fibrinolytic plasma' made by adding partially purified plasmin to normal plasma, and are at present continuing the investigation of the action of plasmin under these conditions.

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Effects on Phosphates of Agents which increase both Respiration and Glycolysis in sections of Cerebral Cortex (Electrical Stimulation, Dinitrophenol, Potassium, Calcium, and Glutamate Salts). By H. McIlwain and M. B. R. Gore. (*Biochemical Laboratories, Institute of Psychiatry, Maudsley Hospital, London, S.E.* 5)

Creatine phosphate is resynthesized in slices of mammalian cerebral cortex during their aerobic metabolism in glucose salines (McIlwain, Buchel & Cheshire, 1951). Substances of the lability of the adenine polyphosphates have now also been found under these conditions. It appeared, therefore, that such slices might be suitable for studying the relationship between phosphorylation and respiration or glycolysis. Few studies have hitherto been made of the levels of phosphates in tissue slices, though slices have very often been used for examining the effects of substances on carbohydrate metabolism.

The following agents which increase respiration and glycolysis in the slices have been found to decrease their content of creatine phosphate (from about 1·4 μmol./g.), usually with increase in inorganic phosphate: electrical stimulation (to 0·6 μmol./g.); the presence of 2:4-dinitrophenol or glutamates (to 0·3 μmol./g.); high K^+ concentrations (to 0·5 μmol./g.); and low Ca^{++} concentrations (to 0·7 μmol./g.). Some fifteen other compounds of various sorts, which did not affect respiration or glycolysis, had negligible effects on the phosphates. High concentrations of some narcotics had a different type of action (Buchel & McIlwain, 1950). Electrical stimulation and dinitrophenol had large effects within 0·5–2 min. of their application.

In interpreting these observations, that relating to electrical stimulation appears the most fundamental. The pattern of changes in the phosphates, respiration and glycolysis is the same as that occurring *in vivo* as a result of increased cerebral activity. It appears likely that such activity is supported by the energy-rich phosphates, and that their breakdown products—inorganic phosphates and phosphate acceptors—accelerate the catabolic processes which restore the energy-rich compounds (see Banga, Ochoa & Peters, 1939; Ochoa, 1941; Long, 1945; McIlwain, 1950). If this is the basic mechanism, operating in the fashion in which it operates in the intact brain, then the other effects may be related to it as follows. One way in which metabolic energy appears to be applied to the electrical changes associated with nervous activity is through maintenance of differential ion concentrations. Increased K^+ and decreased Ca^{++} could increase the metabolic energy necessary for this to be done (see Heilbrunn (1943) on increased ion loss in solutions low in Ca^{++}). Glutamate can affect the transport of potassium (Krebs & Eggleston, 1949) and phosphate, and the dinitrophenol increases inorganic phosphate and phosphate acceptors through inhibiting phosphorylation. 2:4-Dinitrophenol and high K^+ concentrations have been described as agents disturbing the Pasteur effect, which on present views (see Belitzer, 1939; Johnson, 1941; Lipmann, 1942) becomes a special aspect of a general process whereby the level of carbohydrate metabolism is adjusted to meet the varying circumstances of the tissue.

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Acetoin Formation from Pyruvate in *Lactobacillus arabinosus*. By P. M. NOSSAL (1851 Overseas Scholar). (*Department of Biochemistry, The University, Sheffield* 10)

The decarboxylation of pyruvate by washed suspensions of *Lactobacillus arabinosus* 17–5 (Blanchard, Korkes, del Campillo & Ochoa, 1950; Nossal, 1951) shows maximum rates in the presence of $MnCl_2$, KCl and glucose, which substances also accelerate the decarboxylation of L-malate (Nossal, 1951). At equivalent concentrations, $MgCl_2$ is about half as effective as $MnCl_2$, RbCl and CsCl replace KCl, and fructose is somewhat less effective than glucose. The action of glucose is catalytic; its maximum effects occur at glucose concentrations of $0{\cdot}5–2 \times 10^{-3}$M. The optimum pH of the decarboxylation of pyruvate is about 4; CO_2 evolution is more rapid in citrate-phosphate buffer than in acetate buffer; 0·05M-potassium hydrogen phthalate inhibits almost completely. The main product of the reaction (in addition to CO_2) has been identified as acetoin. After heating to 100° for 1 hr. with phenylhydrazine, the reaction product gives the same osazone as acetoin and diacetyl (m.p.'s and mixed m.p.'s 238–242°, corr.). The product differs from diacetyl in that it does not form nickel dimethylglyoxime and that the rate of colour development with α-naphthol, creatine and alkali is slow (Westerfeld, 1945). After oxidation with $FeCl_3$-$FeSO_4$, according to Westerfeld, the distillate gives the reactions of diacetyl. At pH 4, in the presence of glucose, $MnCl_2$ and KCl, the pyruvate utilized is almost completely accounted for by formation of CO_2 and acetoin. In the absence of glucose, formation of CO_2 and acetoin does not account for the pyruvate removal. Glucose, acetaldehyde or lactate alone do not yield acetoin, nor do acetaldehyde or lactate increase acetoin formation from pyruvate. The formation of acetoin from pyruvate in *Lb. arabinosus* therefore shows similarities to that in *Lb. plantarum* (Rowatt, 1949).

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Amine Oxidase and D-Amino-Acid Oxidase in Cephalopods. By H. BLASCHKO and JOYCE HAWKINS. (*Department of Pharmacology, University of Oxford, and the Marine Biological Laboratory, Plymouth*)

Amine oxidase is known to occur in high concentration in the tissues of *Sepia officinalis* (Blaschko, 1941). We have now found that this is also true for *Octopus vulgaris*. Preparations from octopus liver had an enzymic activity higher than similar preparations from mammalian liver; lower, but still very high, activity was found in the posterior salivary glands of octopus; in the anterior salivary glands activity was somewhat lower. We have confirmed earlier observations that tyramine is oxidized much more rapidly than *iso*amylamine by sepia liver. With octopus, however, the ratio oxidation rate with tyramine/oxidation rate with *iso*-amylamine was not so high and was more similar to that found in the guinea pig.

Liver extracts from both octopus and sepia have D-amino-acid oxidase activity. We have chiefly used sepia liver extracts. Oxygen uptakes were found with methionine, phenylalanine, tryptophan, glutamic acid, leucine, norleucine, norvaline, valine and isoleucine. Little or no oxygen uptake was seen with ornithine, lysine, histidine, aspartic acid, proline, serine, threonine, cystine and homocystine, but the rather low activity of some of the extracts together with a high enzyme blank makes these negative results somewhat less certain.

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The Separation of Esters of Choline by Paper Chromatography. By V. P. WHITTAKER and S. WIJESUNDERA. (*Department of Biochemistry, University of Oxford*)

Esters of choline and β-methylcholine have been satisfactorily separated by means of filter-paper chromatography. Visualization of spots has been achieved by the iodine vapour method of Brante (1949) and by a new method specific for carboxylic esters and certain other acyl derivatives. This is an adaptation of a spot test discovered by Feigl, Anger & Frehden (1934) and recently applied to the estimation of acetylcholine by Hestrin (1949). The paper is sprayed with a solution of alkaline hydroxylamine in alcohol (prepared by mixing 10% (w/v) aqueous alcoholic hydroxylamine hydrochloride with 10% (w/v) alcoholic KOH and filtering from KCl); the paper is dried and then sprayed with 3% (w/v) ethereal acid ferric chloride. Carboxylic esters appear as purple spots on a yellow ground. The lower limit of sensitivity with synthetic esters is 0·5–2 μg. Aqueous reagents can also be used (Whittaker, 1951), but the colours are fugitive and there is some diffusion. As acetylphosphate (Lipmann, 1946) and lactones (Kent, 1951) also give colours with this reagent, it may have wider applications. Pharmacological methods of detection have also been used with those esters which cause a contraction of the frog rectus abdominis muscle or guinea pig ileum.

Some R_F values are given in Table 1. *n*-Butanol saturated with water has proved the most generally useful solvent. High water content in a solvent led to diffuse spots and tailing. The R_F values of *n*-acyl cholines and their isomers increase with the number of C atoms and are higher in the more aqueous miscible solvents.

Table 1. *R_F values of choline esters and related compounds*

(All compounds as chlorides.)

Solvent	Choline	Acetyl-choline	Propionyl-choline	Acetyl-β-methyl-choline	Butyryl-choline	Benzoyl-choline
n-Butanol*	0·06	0·09	0·17	0·15	0·22	0·23
Benzyl alcohol-propanol-water (2:5:2)†	0·25	0·33	—	0·37	0·46	0·49
Propanol-formic acid-water (8:1:1)†	0·38	0·46	—	0·56	0·66	0·65

* Descending. † Ascending.

The technique has been applied to the problem of succinyl dicholine hydrolysis. When succinyl dicholine was incubated with horse serum cholinesterase and the solution chromatographed, using benzyl alcohol-propanol-water as the non-aqueous phase, spots identified as succinyl monocholine and choline (identified by the iodine method) made their appearance, but succinic acid was not liberated. This suggests that the initial stage in the breakdown of succinyl dicholine involves the hydrolysis of one ester link and that the enzyme is not able to hydrolyse both choline groups simultaneously. The method is also being successfully applied to the detection of acetylcholine in tissue extracts.

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The Specificity of Cobra Venom Cholinesterase. By L. A. MOUNTER. (*Department of Biochemistry, University of Oxford*)

The aceto-cholinesterases ('true'-cholinesterases) of brain and erythrocytes will hydrolyse a number of aliphatic esters. The rates of hydrolysis of different esters vary considerably: acetates are hydrolysed more rapidly than propionates, while butyrates are almost unattacked. The effect of the alcohol group is determined by the closeness of approach to the choline configuration; thus the optimum is attained with the esters of 3:3-dimethylbutanol, the carbon analogue of choline (Whittaker, 1951). Cobra venom cholinesterase resembles other aceto-cholinesterases in its kinetics and specificity as determined with choline esters and triacetin (Bovet-Nitti, 1947; Blaschko & Holton, 1949; Augustinsson, 1949), and

the effect of this enzyme on various aliphatic esters has now been investigated.

In confirmation of previous work, the kinetics of hydrolysis of acetylcholine and propionylcholine have been shown to give humped Murray-Haldane curves. Acetyl-β-methylcholine and triacetin are hydrolysed, but tributyrin, butyrylcholine and benzoylcholine are not affected by the enzyme.

When sparingly soluble aliphatic esters were added directly to Warburg bottles (0·3 mmol./3 ml.) as in previous specificity studies (e.g. Adams, 1949) the rate of hydrolysis diminished rapidly with increasing molecular weight. Ethyl acetate, propyl acetate and ethyl propionate were hydrolysed at low rates, but butyl and amyl acetates and propionates were scarcely affected. These results differ from those with other aceto-cholinesterases and agree with results obtained by Bovet-Nitti (1947).

Frazer & Stewart (1940) have shown that cobra venom in oil-water emulsions is non-toxic to rabbits due to inactivation of the venom at the oil-water interface. It has been found that this effect can account for the failure of venom cholinesterase to hydrolyse the sparingly soluble substrates. Thus cobra venom is inactivated by shaking with emulsions of water-immiscible substances and the inactivation occurs more readily the greater the degree of dispersion. The addition of certain proteins (e.g. gelatin) or gum acacia is found to protect the enzyme. Using ester emulsions in gum acacia the enzymic rates of hydrolysis of these sparingly soluble esters are much higher than those observed without addition of a protecting agent and are now similar (relative to acetyl-β-methylcholine) to those observed with other aceto-cholinesterases. The specificity pattern under these conditions is similar to that of other aceto-cholinesterases and there is thus no reason for considering the venom enzyme as a separate class of cholinesterase (cf. Zeller, 1948).

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Incorporation of Blood Amino-acids into Milk Proteins in the Rabbit. By P. N. Campbell and T. S. Work. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

Experiments were performed in which ^{14}C-valine labelled in the *gem*-dimethyl group and α-^{14}C labelled lysine were injected intravenously into rabbits during the third week of lactation and the activity of the milk and blood proteins estimated.

Table 1. *Activity of milk and blood proteins (counts/min. in 2 sq.cm. sample of infinite thickness) after administration of* ^{14}C *lysine to a rabbit*

		Time after injection			
		6 hr.	30 hr.	3·5 days	7 days
Blood:	Plasma	60	50	—	17
	Red cells	5	6	—	12
Milk:	Casein	252	45	16	2
	Albumin and globulin	161	33	15	2

The DL-lysine contained 11 μc. of ^{14}C and the DL-valine 6 μc., and they were injected over 1·5 hr. at intervals of 30 min. The young were separated after the last injection and the rabbits milked and bled 6 hr., 30 hr., 3·5 days and 7 days later. The casein was separated from the milk albumin and globulin and the latter were precipitated together by boiling. The blood-plasma proteins were separated from the red cells. The activities of the various fractions are shown in Tables 1 and 2.

Table 2. *Activity of milk and blood proteins (counts/min. in 2 sq.cm. sample of infinite thickness) after administration of* ^{14}C *valine to a rabbit*

		Time after injection			
		6 hr.	30 hr.	3·5 days	7 days
Blood:	Plasma	30	26	—	5
	Red cells	6	12	—	14
Milk:	Casein	333	62	13	4
	Albumin and globulin	253	47	10	4

It will be seen that at 6 hr. the activity of the milk proteins in both cases is very much higher than that of the blood proteins so that it is clear that the free amino-acids of the blood are being incorporated directly into the milk proteins. In the case of the experiment with lysine, the lysine was isolated after hydrolysis of the 6 hr. casein and milk albumin-globulin fractions as its copper benzylidene complex (Turba, 1948). The activity of the isolated lysine was in each case sufficient to account for the whole of the activity of the protein.

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Exogenous and Endogenous Cytochrome *c*. By C. L. Tsou. (*The Molteno Institute, University of Cambridge*)

Added cytochrome *c* is much less catalytically active than the endogenous cytochrome *c*, which is firmly bound to the particles in enzyme preparations (Keilin & Hartree, 1940; Slater, 1950). However, no qualitative difference between them has been demonstrated. In the present investigation it is shown that whereas exogenous cytochrome *c* combines reversibly with cyanide to form a stable complex which resists enzymic reduction (Potter, 1941; Horecker & Kornberg, 1946), the cytochrome *c* present in the Keilin & Hartree (1940) heart-muscle preparation is not affected by prolonged incubation with cyanide.

In spite of very thorough washing with water, the Keilin & Hartree heart-muscle preparation contains sufficient cytochrome *c* for the normal function of the whole cytochrome system. It has now been found that the greater part of this cytochrome *c* can be removed by repeated extraction with salt solutions. A cytochrome *c*-deficient colloidal heart-muscle preparation was obtained by extracting water-washed heart-muscle mince with 0·15M-phosphate buffer, pH 7·3, and then treating the mince in the usual way. This preparation had very little succinic or dihydrocozymase oxidase activity unless cytochrome *c* was added.

When the cytochrome *c*-deficient heart-muscle mince was incubated with a high concentration (3×10^{-5}M) of cytochrome *c* and then followed by the usual procedure, a preparation was obtained which behaved like a normal, cytochrome *c*-containing preparation. The exogenous cytochrome *c* thus incorporated into the colloidal particles was resistant to washing with water, was catalytically as active as endogenous cytochrome *c* and was no longer attacked by cyanide. In other words, it had been transformed into endogenous cytochrome *c*.

The cytochrome *c* obtained in the phosphate extract of water-washed mince had the same activity as the pigment prepared by the more drastic method of Keilin & Hartree (1945), which employs trichloroacetic acid extraction, and was also susceptible to cyanide.

It is therefore clear that: (1) during extraction, endogenous cytochrome *c* is transformed into an exogenous form, which is not only much less active catalytically, but becomes susceptible to the action of cyanide; (2) this transformation is reversible; when the exogenous pigment is again incorporated into the colloidal particles of the heart-muscle preparation by suitable means, it then assumes the characteristic properties of the endogenous form. The proper orientation of cytochrome *c* within a highly specific spatial arrangement of the respiratory pigments is essential for its optimal activity. This orientation seems to be realized through a linkage which binds cytochrome *c* to the colloidal particles of the heart-muscle preparation. When cytochrome *c* is liberated from the colloidal particles by disruption of this linkage (by salt solutions or trichloroacetic acid treatment) it is then capable of reacting with cyanide.

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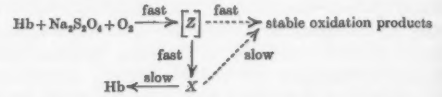
Side-reactions in the Deoxygenation of Dilute Haemoglobin Solutions by Dithionite. By K. Dalziel and J. R. P. O'Brien. (*Department of Biochemistry, Radcliffe Infirmary, Oxford*)

Legge & Roughton (1950) found that an apparent slow terminal stage sometimes occurred in the deoxygenation of sheep haemoglobin solutions by dithionite at neutral pH, and obtained evidence for the formation of abnormal pigments by the action of oxidation products of dithionite, which could invalidate their measurements of the extent of deoxygenation with a Millikan colorimeter employing light filters. The specific cause was not established, but a precursor of choleglobin was suggested; a few experiments in which the composition of the reaction mixture was obtained from the light absorption at 578 mμ. showed no slow phase. In the course of studies of the deoxygenation by dithionite at pH 8·5 of human haemoglobin from normal and diseased persons, with a modified constant flow apparatus (Hartridge & Roughton, 1923) in which the course of the reaction is followed with a Beckman photoelectric spectrophotometer, we found no slow phase in forty kinetic experiments at 430 mμ. and in single experiments at 415, 465 and 560 mμ. Investigations of incidental observations made during this work form the subject of this communication; the results support the conclusions of Legge & Roughton, and throw some further light on the side-reactions involved.

Spectroscopic evidence has been obtained for the initiation of three reactions by the action of oxygen

on dilute solutions of human haemoglobin in dithionite. The net effect is decreased absorption at both haemoglobin maxima, and increased absorption at the 480 mμ. minimum and at 610–640 mμ., and is attributed to the formation of choleglobin and probably other oxidation products. These spectral changes are more pronounced immediately after the reaction with oxygen, but are partially reversed during the subsequent 5 min. Similar slow changes in spectral absorption follow the reduction of oxyhaemoglobin (and methaemoglobin) solutions by dithionite. These observations are attributed to the rapid formation of an unstable compound having isosbestic points with haemoglobin at about 417, 448, 535 and 579 mμ. (pH 8·5) and its subsequent rather slow reversion to haemoglobin. Parallel variation in the extent of the permanent and the transient spectral changes with pH, dithionite concentration and pigment concentration suggests either simultaneous side-reactions or a common intermediate:



Kinetically and spectroscopically, similar reactions follow the addition of small concentrations of hydrogen peroxide to dithionite solutions of haemoglobin, and a transient compound having isosbestic points with haemoglobin in close agreement with those of *X*, and an absorption maximum at 416–418 mμ. (pH 8·3) has been demonstrated. A similar absorption maximum has been obtained by the action of peroxide on dilute catalase-free oxyhaemoglobin solutions.

One of us (K.D.) wishes to acknowledge the receipt of a grant from the Haematology Research Fund.

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Biochemical Tests for Cancer. By P. M. G. Broughton, G. Higgins and J. R. P. O'Brien. (*Department of Biochemistry, Radcliffe Infirmary, University of Oxford*)

Of the many tests advocated for the diagnosis of cancer, three more recent tests based upon quantitative or qualitative changes in serum proteins, seemed worthy of investigation. An assessment of the value of these tests concerned with (*a*) thermal coagulation (Huggins, Miller & Jensen, 1949), (*b*) inhibition of coagulation by iodoacetic acid (Huggins *et al.* 1949), and (*c*) the mucoprotein concentration (Winzler, Devor, Mehl & Smith, 1948), has been attempted upon 32 normal controls, 82 patients with cancer and 48 patients with various non-malignant diseases.

With the thermal coagulation test, 82 % of the malignant cases gave correct positive results and 18 % false negative results, while 63 % of the non-malignant cases gave false positive results.

With the iodoacetic acid test, 55 % of the malignant cases gave correct positive results and 45 % false negative results, while 38 % of the non-malignant cases gave false positive results. There is a tendency for this test to be positive in sera with low protein concentrations, whatever may be the disease of the patient.

The estimation of mucoproteins showed that 73 % of the malignant cases and 47 % of the non-malignant cases had abnormal amounts.

The iodoacetic acid test is therefore the least sensitive of the three tests. Patients with infections usually gave positive results, and, in general, tests were positive in patients with an increased erythrocyte sedimentation rate. No correlation between the site or extent of the lesion or infection with the degree of positivity could be discovered. The three tests gave parallel results; a noticeable exception was cases of cirrhosis which gave positive coagulation tests, but normal mucoprotein concentrations.

It is concluded that (*a*) these changes are in no way specific for cancer, (*b*) the tests are associated with at least two changes—in coagulability and in mucoprotein concentration—which usually occur at the same time.

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DEMONSTRATION

Paper Chromatography of Choline Esters. By S. Wijesundera. (*Department of Biochemistry, University of Oxford*)

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **298th Meeting** *of the Biochemical Society was held in the Department of Physiology, The Medical School, Hospitals Centre, Birmingham* 15, *on Saturday,* 16 *June* 1951.

Election of Sir Charles Martin to Honorary Membership of the Society. Sir Charles Martin, an Original Member of the Society, was elected an Honorary Member. The following appreciation of Sir Charles Martin's contributions to biochemistry and his influence on the development of the subject in this country was presented by Dr W. T. J. Morgan.

'In the course of its history the Biochemical Society has seldom bestowed the distinction of Honorary Membership. W. D. Halliburton, Arthur Harden, Frederick Gowland Hopkins and R. H. A. Plimmer have alone received this honour and to-day your Committee has proposed that Charles James Martin should likewise be elected to Honorary Membership of the Society.

Sir Charles is now in his 85th year and his profound influence on the development of biochemistry during the first 30 years of the present century, although well-known to the older members of the Society, is perhaps less obvious to those of you who have become biochemists in the last two decades.

Sir Charles is an Original Member of the Society and it is therefore appropriate on the occasion of his election to Honorary Membership to recall some of the ways in which he helped to further the progress of our subject in those early years.

C. J. Martin after graduating in London in science and medicine was appointed demonstrator in physiology in the University of Sydney in 1891. His strong and vigorous personality and his ability were soon much in evidence and within the next few years, during which time he became the Professor of Physiology in Melbourne, we find the biochemical aspects of the various problems investigated receiving special emphasis. For example, he studied the combination of diphtheria toxin and of snake venom toxin with their antibodies, and demonstrated that this had taken place by means of ultra-filtration—a new technique which was subsequently to become so important in the first separation by Harden of the coenzymes of fermentation some years later.

In 1903 Martin returned to this country and was appointed the Director of the Lister Institute of Preventive Medicine. He found Arthur Harden installed in the Institute as Chemist and R. H. A. Plimmer was occupying a research studentship in Biochemistry. Martin soon showed himself a man of vision, and encouraged to the full at the Institute the development of biochemistry—or physiological chemistry as it was then called. This activity is perhaps best summarized by mentioning that, between 1903 and 1913, the following persons, many of whom were to become distinguished biochemists later, worked at the Lister Institute: H. D. Dakin, J. B. Leathes, R. H. A. Plimmer, E. P. Cathcart, A. Harden, W. J. Young, P. Hartley, Harriette Chick, Casimir Funk, H. S. Raper, H. Maclean, Ida Maclean, E. L. Kennaway and others. In these early years the main research themes were alcoholic fermentation and fat metabolism, but also important papers on the causation of Beri-Beri, the chemical nature of a substance in rice polishings which cured polyneuritis in birds, the synthesis of 3:4-dihydroxyphenylalanine and other biochemical topics appeared during this period and give some idea of the very varied nature of the biochemical problems being investigated at that time. The Lister Institute may well be proud to have had studying within its walls such a gifted group of workers. This intense biochemical activity was very largely the direct outcome of the Director's enthusiasm and I think it will be difficult to find anywhere in the country at this early date such a large and active biochemical research group.

With the outbreak of World War I nutritional researches occupied many workers in Martin's institute, and among the subjects tackled were the etiology of rickets and its relation to the fat-soluble vitamins, the etiology of pellagra, the multiplicity of the B vitamins and the nature of vitamin C. In a series of investigations commenced in Vienna in 1919 by members of Martin's staff, a very thorough clinical study of rickets was made and convincing evidence was brought forward that rickets could be prevented or cured equally by natural sunlight, artificial ultra-violet radiations or by a vitamin present in cod liver oil. In these early stages of the unravelling of vitamins and accessory food factors it would be fair to say that the results of investigations carried out under Martin's general direction played a very significant part.

There remains yet another major piece of research which owes much to Martin's keen interest in the biochemical aspects of his more purely pathological studies. The return of the workers from Vienna to

this country initiated a detailed experimental study of the pathology of rickets, and Martin became particularly interested to know why cartilage which showed the histological changes preceding ossification had, under normal circumstances, bone salt subsequently deposited in it. Martin was determined to get a biochemist involved in this problem and approached Robison, who was working on fermentation problems in the Lister Institute at that time. Martin's enthusiasm for the subject infected Robison immediately, and in a very short space of time Robison had demonstrated the presence of the enzyme phosphatase in cartilage which was about to calcify and during the years which followed this observation opened up a new field of biochemical study of immense importance.

Many other examples could be given of Martin's profound influence on the development of biochemistry and I am sure that all those who worked with Martin—both here and in Australia, where his influence is held in high esteem—could bring further evidence of the numerous ways in which he showed his faith in biochemistry.

Any account of Martin's influence on the development of biochemistry would be incomplete if it were not to contain a reference to the fact that in 1883, when Martin was a young medical student, he became friendly with Frederick Hopkins who was at that time working as an analytical chemist in the Home Office Expert's laboratory at Guy's Hospital. It is recorded that Martin urged Hopkins to abandon his career as an analyst, study medicine and devote his talents to research in physiological chemistry. This suggestion of Martin's may well have been the impulse which subsequently prompted Hopkins to become qualified in medicine and make the study of biochemistry his life's work.

Although Martin has never claimed to be a biochemist, and indeed for many years he occupied a Chair in Experimental Pathology and made many outstanding contributions to this subject, he nevertheless published numerous papers in the *Biochemical Journal*. He was known to be a competent biochemist and was outstandingly resourceful and inventive in laboratory techniques. I well remember that soon after I joined Martin's institute the research students in the biochemical department received a special warning from their immediate chief Arthur Harden, that if the Director asked a question concerning biochemistry—and he frequently sought the advice of his biochemical colleagues—we must be very careful of our answers for he always thoroughly read up the subject beforehand and generally knew all about it.

Sir Charles retired from the Directorship of the Lister Institute 21 years ago and this year has celebrated the 50th anniversary of his election to the Royal Society.

I feel I cannot conclude these brief remarks without recording the deep debt of affectionate gratitude all members of the biochemical department feel towards their former Chief. His abilities and knowledge are even now, as they always have been, ever at the call of his colleagues. I trust Sir Charles will long be able to enjoy hearing of the progress in the many fields of study he was so instrumental in opening up.

It is my hope that these few notes which I have put together will help to explain why your Committee has put forward the name of Sir Charles J. Martin for election to Honorary Membership of the Biochemical Society.'

COMMUNICATIONS

Inhibition of the Succinic Oxidase System by Fluoride. By E. C. Slater (Australian National University Research Fellow) and W. D. Bonner, jun. (U.S. Public Health Service Fellow). (*Molteno Institute, University of Cambridge*)

The recent finding of Bonner (1951) that phosphate is required for the inhibition of the succinic oxidase system by fluoride indicates a reaction between phosphate and some component of the enzyme system. A reaction of this character must be involved in the esterification of phosphate which accompanies the oxidation of succinate by mitochondrial preparations. Such a reaction with phosphate is not however, essential for the oxidation of succinate (Keilin & Hartree, 1949; Bonner, 1951).

The mechanism of the inhibition of the enzyme system by fluoride and phosphate has been further examined. The degree of inhibition was not affected by the presence or absence of accompanying esterification of phosphate. Thus the system in a mitochondrial preparation was inhibited to the same extent as that in the preparation of Keilin & Hartree (1947), which does not phosphorylate (Table 1). Similarly, the addition of dinitrophenol to the mitochondrial preparation completely prevented the phosphorylation, without affecting the degree of inhibition of the oxidase by fluoride. These experiments suggest that fluoride is not acting on the respiration indirectly by affecting some intermediate in the phosphorylating reaction.

An examination of the degree of inhibition at different succinate concentrations showed that fluoride is a competitive inhibitor of succinic

Table 1. *Inhibition of succinic oxidase system by* NaF *in both phosphorylating and non-phosphorylating systems*

(1·82 mg. (fat-free dry wt.) pig heart mitochondria (M); 1·91 mg. horse-heart Keilin-Hartree preparation (K-H). Phosphate, pH 7·3, 0·0348 M; succinate, 0·0267 M; cytochrome *c*, $5{\cdot}44 \times 10^{-5}$ M in all flasks. Those provided with 'adenylic system, etc.' contained, in addition, adenylic acid, $1{\cdot}6 \times 10^{-3}$ M; ADP, $0{\cdot}63 \times 10^{-3}$ M; ATP, $0{\cdot}29 \times 10^{-3}$ M; glucose, 0·083 M; $MgCl_2$, 0·047 M and hexokinase. Dinitrophenol (DNP), 5×10^{-4} M, and NaF, 0·04 M, where stated. Total volume, 3 ml. Time of experiment, 30 min. Temperature, 25° with M; 38° with K-H preparation. Δ HP (hexosemonophosphate) measured as described by Slater (1950).)

Preparation	Adenylic system, etc.	DNP	NaF	O_2 uptake (μatoms)	Δ HP (μmol.)	P:O	Inhibition of O_2 uptake by NaF (%)
M	−	−	−	9·51	−	−	62
M	−	−	+	3·58	−	−	
M	+	−	−	9·91	10·5	1·03	59
M	+	−	+	4·07	3·7	0·91	
M	+	+	−	9·56	0·04	0	54
M	+	+	+	4·44	0	0	
K-H	−	−	−	24·0	−	−	58
K-H	−	−	+	10·0	−	−	

dehydrogenase. In the absence of fluoride, phosphate is itself a weak competitive inhibitor. This suggests the following mechanism:

$$(1)\quad E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES$$

$$(2)\quad ES \xrightarrow{k_3} \text{products} \qquad K_m = \frac{k_2 + k_3}{k_1},$$

$$(3)\quad E + P \underset{k_5}{\overset{k_4}{\rightleftharpoons}} EP \qquad K_P = \frac{k_5}{k_4},$$

$$(4)\quad EP + F \underset{k_7}{\overset{k_6}{\rightleftharpoons}} EPF \qquad K_{PF} = \frac{k_7}{k_6},$$

where E is succinic dehydrogenase, EP and EPF are the inactive phospho- and fluorophospho-enzyme complexes, respectively, and ES is the active enzyme-substrate compound. This mechanism gives

$$v = \frac{V_{max}}{1 + \frac{K_m}{s}\left(1 + \frac{p}{K_P} + \frac{pf}{K_P K_{PF}}\right)},$$

where p, f and s are the concentrations of phosphate, fluoride and succinate respectively. If this mechanism is correct, the experimental data suggest that the dissociation constants are of the following order of magnitude: K_m, $4{\cdot}8 \times 10^{-4}$ M; K_P, 0·1 M; K_{PF}, $6{\cdot}7 \times 10^{-5}$ M.

Reaction (3) might possibly be one of the reactions leading eventually, in the complete system, to the esterification of phosphate.

The degree of inhibition by phosphate and fluoride is not affected by the addition of magnesium.

Arsenate inhibits like phosphate, but to a smaller degree.

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The Lipids of Cell Nuclei Isolated from Human Brain Cortex. By L. W. Tyrrell and D. Richter.
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Cell nuclei were isolated in quantity from adult human brain cortex by the method of Richter & Hullin (1951). The lipids were determined in chloroform-ethanol (1:1) extracts of the trichloroacetic acid precipitates prepared from the whole tissue and from cell nuclei. Approximate figures for the composition of the cytoplasm of the cells were calculated on the basis of the estimated value for the proportion of the whole tissue contributed by the cell nuclei.

The lipid extracts were taken to dryness under reduced pressure at 60° and total phospholipin P, monoaminophospholipin P and sphingomyelin P determined after hydrolysis of the residues with N-NaOH at 37° (cf. Schmidt, Benotti, Hershman & Thannhauser, 1946). Phosphorus was determined colorimetrically by the method of King (1932), and a factor of 25 was used to convert phosphorus values to phospholipin. Lecithin was determined by estimating the choline released during the alkaline

hydrolysis, by the reineckate method of Glick (1944). An estimate of cephalins was obtained by difference from the monoaminophospholipin and lecithin figures. Total cholesterol was determined absorptiometrically by the Liebermann-Burchard reaction; cerebrosides were determined by the method of Sperry & Brand (1941).

The results (see Table 1) showed that all of the lipids investigated were present in the isolated cell nuclei as well as in the whole brain cortex. The cerebroside concentration in the cell nuclei was over 100% higher than in the whole tissue; other lipids were present in relatively lower concentration. In the phospholipin fraction of the lipids of the cell nuclei the proportions of monoaminophospholipins, sphingomyelins, lecithins and cephalins were similar to those in the whole tissue.

Table 1. *Lipids in whole tissue, isolated cell nuclei and cell cytoplasm, expressed as percentage of dry tissue weight in human brain cortex*

(Values given represent the means for five preparations)

	Whole cortex	Nuclei	Cytoplasm (calculated)
Total cholesterol	5·3	4·1	5·4
Total phospholipins	19·7	13·0	20·3
Monoaminophospholipins	16·8	10·9	17·3
Sphingomyelins	2·9	2·1	3·0
Lecithins	5·2	4·4	5·4
Cephalins	11·6	6·5	11·9
Cerebrosides	4·8	10·0	4·7

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The Metabolism of Benzene. The Nature and Amount of the Muconic Acid in Benzene Urine.

By D. V. Parke and R. T. Williams. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*)

One of the metabolites of benzene in animals is muconic acid (hexa-2:4-diene-1:6-dioic acid). On stereochemical grounds it would be expected that benzene would give rise to *cis-cis*-muconic acid, but in the past, seven groups of workers have reported the isolation of the *trans-trans*-acid from urine. It has been suggested that the *trans-trans*-acid is an artifact produced by isomerization of the *cis-cis*-acid. Recently, Elvidge, Linstead, Sims & Orkin (1950) have shown that the *cis-cis*-acid is readily isomerized to the *cis-trans*-acid by mere recrystallization from water, but the isomerization to the *trans-trans*-acid requires a catalyst and is not carried out by heat alone.

We have found that on heating the *cis-cis*- or *cis-trans*-acids at 100° for 4–8 hr. with an excess of phenol and a little conc. H_2SO_4, a crimson colour is obtained ($\lambda_{max.} = 520$ mμ.). The *trans-trans*-acid gives no colour under these conditions, but does so if the temperature is raised to 150–160°. The *cis-cis*- and *cis-trans*-acids can readily be distinguished from the *trans-trans*-acid by this means. The colour reaction has also been put on a quantitative basis and the output of muconic acid estimated.

Ether extraction of benzene urine from rabbits under conditions which do not convert the *cis-cis*- and *cis-trans*-acids into the *trans-trans*-acid yielded only *trans-trans*-muconic acid (m.p. 300° (decomp.); benzhydryl ester m.p. and mixed m.p. 190°). Estimations of the acids by the colorimetric method developed and under conditions where the urine was not subjected to heat showed that at a dose level of benzene of 0·5 g./kg. an average of 0·6% of the benzene was excreted as *trans-trans*-muconic acid. Crude muconic acid extracted from urine was tested for us by Dr W. C. Evans, Aberystwyth, using a microbiological test which distinguishes the *cis-cis*-acid from the other two isomers (Evans & Smith, 1951). No *cis-cis*-muconic acid was found in our sample.

Our present observations lead us to the conclusion that the muconic acid present in the urine of rabbits dosed with benzene is the *trans-trans*-isomer and that if this compound is formed from the *cis-cis*-acid, then the isomerization or some other mechanism of transformation takes place *in vivo*.

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Nitrogen Metabolism and Carotenogenesis in *Phycomyces blakesleeanus*. By T. W. GOODWIN, W. LIJINSKY and J. S. WILLMER. (*Department of Biochemistry, University of Liverpool*)

Valine and leucine are the only two naturally occurring amino-acids containing a terminal *gem*-dimethyl group; they can therefore, theoretically, give rise to the postulated 5-carbon building units (e.g. β-methylcrotonaldehyde) for carotene synthesis. In media containing adequate amounts of glucose (10% w/v), these amino-acids, when used as the sole nitrogen source, have no stimulatory effect when compared with other amino-acids such as asparagine, glycine, or alanine (Garton, Goodwin & Lijinsky, 1951). It has now been found that on media containing glucose in amounts insufficient for maximal carotene production, but sufficient for reasonable mycelial growth (1% w/v), valine and leucine do specifically stimulate carotene synthesis compared with the other amino-acids; in some cases stimulation was as much as 400%. It thus appears very probable that valine and leucine can provide the repeating unit for carotene synthesis; in the presence of excess glucose this effect is not observed because glucose itself can provide adequate amounts of the building unit.

Concurrent measurements, at various stages of growth, of nitrogen assimilation and carotene production by *Phycomyces*, growing on a glucose-asparagine medium, have shown that most of the carotene produced is synthesized only after nitrogen assimilation has ceased. This agrees with the previous observation that growth (as measured by dry weight production) must be complete before carotenogenesis is maximal (Garton *et al.* 1951).

The observation of Garton *et al.* (1951) that fully grown mycelia transferred to media containing glucose, but no nitrogen, did not synthesize carotene, but did so if transferred to media containing both glucose and nitrogen, has now been shown to be due to the pH conditions of their media, which were buffered at pH 6·7, and not to the suggested possibility that the fungus must be metabolizing exogenous nitrogen in order to synthesize carotene. If the replacement media are at pH 6·7, but not buffered, carotene synthesis proceeds both in the presence and absence of nitrogen, but is very much greater in its presence.

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Further Observations on Ethereal Sulphate Formation *in vivo*. By H. G. BRAY and K. WHITE. (*Department of Physiology, The Medical School, University of Birmingham*)

We have previously reported on the kinetics of the conjugation of phenols in the rabbit (Bray & White, 1951). In the fasting animal the ethereal sulphate conjugation of an administered phenol follows zero-order kinetics, the rate being limited by the availability of sulphate ion. For most phenols studied, simultaneous administration of sufficient sulphate precursor (e.g. Na_2SO_3, cystine), leads to a first-order ethereal sulphate conjugation with respect to the phenol. With *p*-hydroxybenzenesulphonamide and *p*-hydroxybenzamide, however, the reaction still follows zero-order kinetics even after administration of the maximum dose of a sulphate precursor which can be safely tolerated by the animal.

Since a general formulation of the kinetics of ethereal sulphate formation must relate the reaction rate to concentrations of both phenol and sulphate, the scope of the investigation has been extended by the analysis of blood as well as of urine.

In experiments performed so far, it has been found that the blood level of sulphate ion is raised by the administration of a sulphate precursor and lowered by a phenol. When both are given together, the blood sulphate level is raised slightly.

The blood level of ethereal sulphate does not appear to change, within the limits of experimental error, even when ethereal sulphate is being rapidly excreted.

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The Metabolism of Anisole and Diphenyl Ether in the Rabbit. By H. G. Bray, W. V. Thorpe and Marie R. Wasdell. (*Department of Physiology, The Medical School, University of Birmingham*)

Unsubstituted aromatic ethers of the type $C_6H_5.OR$ possess no group which can be directly conjugated in the animal body. It was, therefore, of interest to discover whether the ether link could be hydrolysed *in vivo* giving rise to a phenol which could then be conjugated with glucuronic acid or sulphuric acid, or whether conjugation was more readily effected by introduction of new hydroxyl groups. Splitting of the ether link has been demonstrated in the substituted ethers, *p*-phenetidine and its *N*-acetyl derivative, phenacetin (Smith & Williams, 1949), but Kossel (1880, 1883) and Lehmann (1889) found that phenetole itself was hydroxylated without the ethoxy group being changed.

When anisole (0·5 g./kg.) is administered to rabbits it is excreted mainly conjugated with glucuronic acid and sulphuric acid (48 and 29 % of dose respectively). About 4 % of the dose is excreted as free phenol. The chief metabolite in the urine has been identified as *p*-methoxyphenol. Small amounts of *o*-methoxyphenol are formed, but no phenol is excreted. Diphenyl ether (dose 0·5 g./kg.) is also excreted, mainly conjugated with glucuronic acid and sulphuric acid (63 and 12 % of dose respectively). *p*-Hydroxyphenyl phenyl ether has been isolated from urine, but no phenol was detected. A small amount of a phenol which is probably bis (*p*-hydroxyphenyl) ether has also been isolated; *o*-hydroxydiphenyl phenyl ether was not found.

The evidence as present available suggests that the group —OR in unsubstituted aromatic ethers cannot act as a 'potential centre for conjugation' (Bray, Ryman & Thorpe, 1948). Such compounds are conjugated by the formation of a new centre for conjugation by hydroxylation.

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An Examination of Frog Jelly. By Sybil P. James. (*Department of Physiology, The Medical School, University of Birmingham*)

A preliminary examination of the sugars produced by hydrolysis of the jelly-like mucin of frog spawn (Bray & James, 1949) showed the presence of fucose, galactose and amino sugars. Glucosamine and chondrosamine were subsequently identified. Only part of the amino sugar reacted with sodium carbonate (0·05 N). Folkes, Grant & Jones (1950) showed that these four were the major sugar constituents, and also found a smaller amount of mannose and traces of glucose and xylose.

Attempts have been made to determine the way in which the sugars are linked. The complex prepared from frog spawn mucin by the method of Giacosa (1882) was methylated until a product with maximum methoxyl content was obtained. From the hydrolysed methylated product 2:3:4-trimethylfucose, 2:3:4:6-tetramethylgalactose and 2:4:6-trimethylgalactose were isolated. The identification of another methylated sugar, probably a dimethylgalactose, has not been completed. Several amino-acids (aspartic acid, glutamic acid, glycine, threonine), but no amino-sugar derivatives, were detected in the hydrolysate.

These results indicate that in the methylate, the fucose and some galactose units are linked to the main structure through $C_{(1)}$ only, other galactose units are linked through $C_{(3)}$, and yet others may be joined through two positions not yet determined.

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The Liberation of Chloride Ions from Organic Chloro-Compounds by Tissue Extracts. By H. G. Bray, W. V. Thorpe and D. K. Vallance. (*Department of Physiology, The Medical School, University of Birmingham*)

The liberation of chloride ions from organic chloro-compounds by tissue extracts has been investigated. The livers of rabbit, rat and guinea pig were appreciably active in this respect, and the behaviour of rabbit liver towards organic chloro-compounds has been examined. Butyl chloral hydrate, chloral hydrate, trichloroacetamide, ω-chloroacetophenone, pentachloroethane and hexachloroethane (at a concentration of 0·024 M, and at pH 6·8) were readily decomposed, and underwent little or no spontaneous hydrolysis. The active factor is only partially destroyed by prolonged boiling, and is not completely dialysable. The amount present in the liver of intact animals appears to be depleted as a result of injections of chloral hydrate.

It is suggested that the reaction is not primarily enzymic, but may consist fundamentally of the interaction between sulphydryl groups and chlorine atoms of the organic compound. This hypothesis was tested by investigating the concomitant changes in chloride and sulphydryl content of digests containing sources of sulphydryl groups (tissue extract and/or cysteine, glutathione or thioglycollic acid) and various chloro-compounds (chloral hydrate, butyl chloral hydrate and trichloroacetamide). In several instances there was a quantitative relationship between disappearance of sulphydryl groups and appearance of chloride ions which was in agreement with a reaction

$$R—SH + X—Cl \rightarrow R—S—X + H^{\cdot} + Cl'.$$

This finding is not entirely in agreement with the claim of Heppel & Porterfield (1948) to have discovered an enzyme in rat tissues which can liberate inorganic chloride from aliphatic chlorinated hydrocarbons.

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Biosynthesis of Globin in the Rat. By Helen M. Muir, A. Neuberger and J. C. Perrone. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

Radioactive glycine and valine labelled with ^{14}C on the methylene and *gem*-methyl groups respectively were injected into groups of rats, and the animals killed after suitable intervals (12, 24, 72 hr.; 1 and 2 weeks). The doses were 5 μc. glycine/100 g. body weight and 3·5 μc. DL-valine/100 g. body weight. Rat haemoglobin, like the haemoglobin of other species, contains terminal amino groups derived from valine. Valine was therefore isolated as the 2:4-dinitrophenyl derivative (Perrone, 1951) from terminal and non-terminal positions of the crystalline haemoglobin. Glycine was isolated from the globin and also from the 'stromatin' (ghosts). The porphyrin was obtained as protoporphyrin dimethyl ester. The comparison between the radioactivities of the terminal and non-terminal valine (Table 1) show clearly that these are identical for each point in time. Allowing for the differences in radioactive dosage the ratios of the molecular specific radioactivities of the isolated glycine and valine derivatives were calculated and found to be 1·2 ± 0·2. This result suggests that the glycine and valine 'pools' and their respective turnover rates are somewhat similar.

Table 1. *Radioactivities of valine samples from rat globin*

(V_E = terminal valine; V_m = non-terminal valine. Results are given in counts/min./sq.cm. (infinite thickness).

Time of collection	V_E	V_m
24 hr.	33·1 ± 1·9	33·18 ± 1·58
72 hr.	74·19 ± 3·49	76·05 ± 3·06
1 week	58·12 ± 1·78	59·45 ± 1·18
2 weeks	102·67 ± 6·23	103·04 ± 3·63

The constancy of these two ratios suggests that the different peptide linkages in haemoglobin are formed over a time interval, which is relatively short compared with the rather rapid decline in isotope content in the free amino-acids in the body. The findings are compatible with the hypothesis that globin synthesis consists either of a simultaneous condensation of amino-acids or of a rapid successive formation of peptide bonds without any appreciable accumulation of intermediates.

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End Products of the Action of Salivary α-Amylase on Amylose. By P. J. P. ROBERTS and W. J. WHELAN. (*Department of Chemistry, University College of North Wales, Bangor*)

Potato amylose (prepared as by Hobson, Pirt, Whelan & Peat, 1951) is hydrolysed by purified salivary α-amylase (prepared by the method of Meyer, Fischer, Staub & Bernfeld, 1948) to a mixture of reducing sugars having a reducing value equivalent to 90·0% apparent maltose. The action ceases abruptly at this point. The end products of the action have been prepared on a large scale and have been fractionated on a charcoal column (Whistler & Durso, 1950; Bailey, Whelan & Peat, 1950). They are found to consist exclusively of maltose and maltotriose in the molecular proportion 2·39:1. A mixture of the two sugars in this proportion has a reducing value which is equivalent to 88·9% maltose. From 2·708 g. of amylose were obtained 1·623 g. of pure maltose (anhydrous) and 0·999 g. of pure maltotriose. Allowing for a loss of amylose of 6·48%, due to precipitation by the enzyme, the recovery is 93·1%. Neither glucose nor maltosaccharides higher than the triose were detected. Salivary α-amylase was entirely without action on the separated maltose or maltotriose, which therefore represent the final products of the amylase action. In this respect salivary α-amylase differs from pancreatic amylase, which has been found by Meyer & Gonon (1951) to hydrolyse maltotriose to maltose and glucose. On the assumption that α-amylase fragments the polysaccharide chains by random scission, giving rise to maltose and maltotriose, and then hydrolyses the latter sugar slowly to glucose and maltose, these authors have shown that a statistical calculation of the final yield of glucose agrees closely with the experimental yield. Their calculation indicates that before hydrolysis of the maltotriose the molar ratio, maltose:maltotriose, should be 2·35:1. It is significant that this ratio agrees well with that which we have determined experimentally, namely, 2·39:1.

The analytical data for the isolated end products are given in Table 1. They are based on the amounts of glucose liberated by acid hydrolysis (method of Pirt & Whelan, 1951). Reducing and R_F values are expressed as percentages of the corresponding values for authentic samples of maltose and maltotriose.

Table 1. *End products of the action of salivary α-amylase on amylose*

	$(\alpha)_D^{15°}$	Reducing value*	R_F value†
Maltose (anhydrous)	+136°	98·3	100
Maltotriose	+160°	99·3	100

* Method of Somogyi (1945).

† In fusel oil-pyridine-water (Jeannes, Wise & Dimler, 1951).

The maltotriose is hydrolysed by crystalline and purified soya bean β-amylases to maltose and glucose. The reducing values obtained after β-amylolysis were 100·7 and 101·0%, respectively, of the calculated values.

Similar studies are now in progress to determine the end products of α-amylase action on glycogen and amylopectin.

The authors wish to thank Prof. S. Peat, F.R.S., for his advice and encouragement

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Selective Inhibition of Enzymic Impurities Associated with Potato Phosphorylase. By J. M. BAILEY, GWEN J. THOMAS and W. J. WHELAN. (*Department of Chemistry, University College of North Wales, Bangor*)

Synthetic amyloses of chain lengths up to 1000 glucose units have been prepared using potato phosphorylase (*A*) isolated by lead complex precipitation (Barker, Bourne, Wilkinson & Peat, 1950), and further ammonium sulphate fractionation as in Hobson, Whelan & Peat (1950). These polysaccharides differ from natural potato amylose in that the blue values (B.V.) (Bourne, Sitch & Peat, 1949) and wavelengths of peak absorption ($\lambda_{max.}$) of the iodine-stained synthetic amyloses never exceed 1·2 and 620 mμ. respectively, whereas the respective values for natural amylose are 1·45–1·50 and 645 mμ. It would appear that the phosphorylase is contaminated by either α-amylase

or Q-enzyme, both of which lower the B.V. and $\lambda_{max.}$ of amylose. In the presence of mercuric chloride, which completely inhibits α-amylase and Q-enzyme (Sitch, 1948), but causes only weak inhibition of phosphorylase, the phosphorylase preparation causes the synthesis from glucose-1-phosphate of a polysaccharide which is identical with natural amylose in respect of the above properties. Whilst the amylolytic activity of phosphorylase A is so slight as not to be detected by the usual methods, it has been shown that the readily detectable amylolytic activity of a potato phosphorylase (B) prepared as in Bourne *et al.* (1949) is also completely suppressed by mercuric chloride.

A second enzymic impurity present in phosphorylases A and B to an equal extent with respect to phosphorylase activity, is a phosphatase which hydrolyses glucose-1-phosphate and glucose-6-phosphate. The phosphatase action gives rise to erroneous estimates of chain length during amylose synthesis, because the chain length is calculated from the amount of phosphate ion liberated. The use of molybdate ion as a phosphatase inhibitor was suggested by Swanson (1950), and we have found that this ion completely inhibits potato phosphatase, but has no action on phosphorylase. In the presence of molybdate and absence of added 'primer', phosphorylase A causes not the slightest liberation of phosphate from glucose-1-phosphate during 24 hr. or more at 35°. Polysaccharide synthesis may be initiated at any time during this period by the addition of primer.

It has been further observed that phosphoglucomutase and isomerase are present in phosphorylase B, but not in phosphorylase A. Mercuric chloride causes complete inhibition of the phosphoglucomutase, but of all the above enzymes only phosphatase is completely inhibited by molybdate.

The amounts of mercuric chloride and ammonium molybdate necessary for the complete inhibition of amylolytic and phosphatase actions in phosphorylase A are, respectively, $1{\cdot}54 \times 10^{-8}$ and $1{\cdot}02 \times 10^{-4}$ moles per Green & Stumpf (1942) unit of phosphorylase.

The authors wish to thank Prof. S. Peat, F.R.S., for advice and encouragement.

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Aldehyde Mutase and the Dismutation of Formaldehyde. By L. P. KENDAL and A. N. RAMANATHAN. (*Department of Physiology, University of Manchester*)

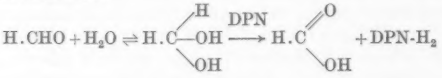
The behaviour of formaldehyde (0·5 mg./ml.) alone, and in the presence of methanol (5 mg./ml.), as a substrate of horse-liver aldehyde mutase (Dixon & Lutwak-Mann, 1937) has been studied. Acid production was followed manometrically in the Warburg apparatus, using a coenzyme I (Co I) and bicarbonate-containing medium with 95 % N_2 + 5 % CO_2 as the gas phase, at pH 7·4 and 37°. Residual formaldehyde was estimated by a colorimetric method due to Leaf & Zatman (unpublished) and based on a method of Wright (1927), and formate was estimated by the procedure of Bastrup (1947).

In the absence of added methanol, formaldehyde disappeared and acid was formed in an approximately 2:1 molar ratio, in accordance with the expected dismutation. Chemical and manometric estimations of formate were in reasonable agreement.

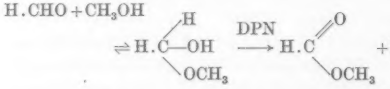
The further introduction of methanol into the system accelerated the disappearance of formaldehyde, but had no corresponding effect upon acid production as judged by manometric observation. In the early stages the ratio [formaldehyde used]/[acid formed] was >4. Acid production continued after the chemical test indicated complete disappearance of formaldehyde, and in a prolonged incubation finally reached a figure concordant with classical dismutation theory. Chemical estimation of formate, in contrast, indicated no such lag of formate formation behind formaldehyde disappearance, and the ratio [formaldehyde used]/[formate formed] was approximately 2 throughout.

The isolation of an aldehyde dehydrogenase from liver mutase preparations by Racker (1948) has increased the probability that mutase is a coenzyme-linked system with aldehyde- and alcohol-dehydrogenase components. The above experiments sug-

gested the possibility that in the presence of methanol the reactions:



were largely replaced by



the semi-acetal competing successfully with the hydrate for the enzyme, and undergoing a more rapid dehydrogenation. The primary product, methyl formate, would then yield formic acid only by a comparatively slow hydrolysis, but is not distinguished from free formic acid in estimations by Bastrup's method.

In experiments at room temperature a stream of N_2 was passed through a system containing enzyme, Co I, formaldehyde and methanol and maintained at pH 7·4, into a trap containing 0·2N-NaOH. The greater part (> 70 %) of the total formate produced was then shown to have been carried over into this trap, and must therefore have been in a form volatile from solution at pH 7·4, and behaving in this respect like methyl formate.

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Some Investigations on Acid-soluble Serum Proteins. By DEIRDRE M. WALDRON (introduced by D. L. WOODHOUSE). (*Cancer Research Laboratories, The Medical School, Birmingham* 15)

The acid-soluble mucoproteins of serum have been measured in the past by means of polarographic 'filtrate wave' test of Brdicka, Novak & Klumpar (1939). This test was proposed as a diagnostic aid for cancer, but has been shown to be positive in many other pathological conditions. The rise in concentration of the mucoprotein does not appear to be dependent on tissue necrosis, but it is usually accompanied by an increase in α_1- and α_2-globulins. The isolated material migrates with α_1-globulin when added to serum.

In an attempt to trace the source of the mucoprotein a thorough qualitative analysis of its component parts has been undertaken. Winzler, Devor, Mehl & Smith (1948) published the following figures for an acid-soluble protein: hexose 15·1 %, hexosamine 11·9 %, and total nitrogen 7·9 %. By paper chromatography, the hexoses have been identified as galactose and mannose. A further component with the R_F value of fucose has also been observed. Galactose is readily released by acid hydrolysis, while mannose and fucose are more resistant. Fucose measured by the Dische & Shettles (1948) cysteine reaction was between 1 and 2 % of the material. The hexosamine was detected after mild hydrolysis as the *N*-acetyl derivative by spraying with Morgan & Elson reagent, and identified as glucosamine by oxidative degradation to arabinose on heating with ninhydrin after the method of Gardell, Heijkenskjold & Rochnorlund (1950).

The results of a quantitative amino-acid analysis carried out by the methods of Moore & Stein (1948, 1949) correspond to a more basic protein than that described by Weumer, Mehl & Winzler (1950) for their salt-fractionated mucoprotein.

When subjected to electrophoresis at pH 4·5, the acid-prepared mucoprotein can be shown to consist of three components. The mucoprotein prepared by Weumer *et al.* (1950) by salt fractionation has been identified as the component with the lowest isoelectric point. This would account for the more basic character of the acid-prepared mucoprotein.

The relationship between serum 'trypsin inhibitor' and mucoprotein is being investigated. The values obtained by polarographic analysis and the inhibition by diluted serum of the activity of crystalline trypsin show a direct relationship, with a fair amount of scatter. Activity is found in certain fractions of serum soluble in 50 % saturated ammonium sulphate at pH 5·1.

The inhibitory activity of isolated mucoprotein prepared by salt fractionation is proportional to its concentration and can account for one-twenty-fifth of the activity of serum. The relatively high concentration of mucoprotein necessary to inhibit a given quantity of trypsin suggests that the activity is due to a minor component of the material.

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Metabolism of Lower Fatty Acids by Sheep Rumen Epithelium. By R. J. Pennington. (*The Rowett Research Institute, Bucksburn, Aberdeenshire*)

Lower fatty acids, particularly acetic, propionic and butyric acids, formed by bacterial action in the rumen of the sheep, are absorbed in considerable quantities from this organ (Barcroft, McAnally & Phillipson, 1944). It was further observed (Masson & Phillipson, 1951) that the relative concentration of the individual acids in the blood leaving the rumen did not parallel the rates at which they disappeared from the rumen. The differences indicated that butyrate, at least, was metabolized to some extent in the wall of the rumen itself. To investigate this further, solutions of the sodium salts of each of the three fatty acids in Krebs's Ringer-phosphate solution (pH 7·4) were incubated aerobically with rumen epithelial tissue and the changes in their concentration measured. All three disappeared to some extent, but there was invariably a much greater loss of butyrate than of the other two. The utilization of propionate was greatly enhanced if an atmosphere containing 5 % carbon dioxide was used.

The disappearance of butyrate was accompanied by the production of ketone bodies in quantities sufficient to account for more than one-half of the butyrate lost; the proportion of β-hydroxybutyric acid in the ketone bodies formed was low. Ketone bodies were found also after incubation of acetate, but not of propionate; the latter appeared to exert an antiketogenic effect. Such a marked production of ketone bodies by tissues other than liver tissue is very unusual and may have a special significance in the metabolism of ruminants.

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Attempts to Detect Growth Hormone in Samples of Blood and Urine Obtained During Pregnancy or Lactation. By P. Mary Cotes and F. G. Young. (*Biochemical Laboratory, University of Cambridge*)

During pregnancy (Cole & Hart, 1938) or lactation (Young, 1950) in the rat, the mother herself increases in weight. Watts (1935) found that growth-promoting anterior pituitary extracts, administered to female rats during pregnancy, produced extra growth in the mother and heavier litters. Cotes, Crichton, Folley & Young (1949) showed that purified preparations of growth hormone were galactopoietic in the cow in declining lactation. These observations might be correlated if the influence of growth hormone is a factor common to the metabolic changes associated with growth, including foetal growth, and milk production.

Attempts have now been made to detect growth hormone in blood from lactating women and from a pregnant goat, and in urine samples from pregnant and lactating women. Growth hormone has been assayed in hypophysectomized female rats by the tibial test of Greenspan, Li, Simpson & Evans (1949). In our hands, a total dose of 10 μg./rat could be detected by this method.

Human blood, from the median basilic vein, was drawn into cooled centrifuge cups containing heparin, and the plasma was separated by centrifugation. A globulin fraction of the plasma protein, precipitated by addition to the plasma of an equal volume of saturated ammonium sulphate solution, was dialysed free of salt and freeze-dried. Samples of these globulin fractions, equivalent to a total dose of 6–10 ml. plasma per rat, were then tested for growth hormone activity. Growth hormone added to whole blood has been partially recovered by this method. 24 hr. samples of urine, preserved with merthiolate, were collected and sodium bicarbonate

added to bring the pH to about 7. Globulin fractions, precipitated by half-saturation with ammonium sulphate, were tested for growth-hormone activity.

Under our experimental conditions, no growth hormone has so far been detected in fractions from samples of blood and urine obtained during pregnancy or lactation.

We are indebted to Dr N. R. Lawrie and to Dr G. R. S. Grogono for help in obtaining physiological samples and to the Medical Research Council for their financial support.

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A Citrate Oxidation Factor from Yeast. By E. C. Foulkes (Australian National University Scholar).
(*Department of Biochemistry, University of Oxford*)

Recent experiments (Foulkes, 1951) have suggested that in cell-free preparations from baker's yeast, citrate is oxidized by two different mechanisms: the first is related to the normal tricarboxylic acid cycle of mammalian tissues and leads to α-ketoglutarate; the second requires adenosinetriphosphate (ATP), Mg^{++}, and an unidentified dialysable factor present in boiled yeast juice. This citrate oxidation factor (COF) has now been considerably concentrated and purified, and its function has been studied. It is not identical with the pyridine nucleotides or coenzyme A. COF forms no insoluble salts with Ba^{++}, Hg^{++}, or basic lead acetate. Judging from its behaviour in various solvents, it appears to be non-ionized in alkaline solvents, but ionized at acid pH. The purest preparations contain traces only of phosphate. Pyrimidine pentosides are present in the final concentrate. The process of purification involves removal of inactive material with barium and ethanol, extraction with phenol, treatment with anionic ion-exchange resin and partition chromatography on cellulose using ammoniacal *n*-propanol (70%)–water (30%) (v/v). The assay system consists of the dialysed cell-free enzyme preparation previously described (Foulkes, 1951), to which are added substrate (citrate), ATP, Mg^{++} and COF. The purified concentrate contains less than 10 μg. pentose, and 0·05–0·1 μg. P per optimum dose. The latter is presumably a trace impurity. The relative absence of phosphate, as well as non-precipitation with basic lead acetate, distinguish COF from the uridinediphosphate-glucose isolated from yeast by Caputto, Leloir, Cardini & Paladini (1950). As yeast nucleic acid, hydrolysed to the nucleoside stage by the technique of Bredereck, Martini & Richter (1941) is completely inactive, and as COF is inactivated by *N*-acid or *N*-alkali at 100° C., the structure of the molecule appears to be more complex than that of a simple nucleoside.

In the presence of semicarbazide, 1 mol. of CO_2 is formed for each mol. of citrate metabolized, but no α-ketoglutarate is produced. The recent data of Ajl & Kamen (1951) on the oxidation of acetate by *Escherichia coli* also possibly suggest a pathway of citrate breakdown which does not proceed via α-ketoglutarate.

Boiled brain juice possesses some activity, and present work is concerned with the behaviour of COF in mammalian tissues, as well as with the detailed structure and function in the yeast preparation.

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The Oligosaccharides Synthesized by *Escherichia coli* from Maltose. By S. A. Barker and E. J. Bourne (introduced by M. Stacey). (*Chemistry Department, University of Birmingham*)

Monod & Torriani (1948) isolated from *Escherichia coli* (cf. Monod, Torriani & Gribetz, 1948) an enzyme, amylomaltase, which utilized maltose as a substrate in the synthesis of starch-type dextrins. The reaction was shown to conform with the equation

$$n \text{ maltose} \rightleftharpoons n \text{ glucose} + (\text{glucose})_n.$$

Doudoroff, Hassid, Putman, Potter & Lederberg (1949) reported a study of the metabolism of maltose by a suspension of a mutant of *Esch. coli* (W–327), which, in contrast to Monod's strain, was incapable of utilizing lactose or glucose. They were able to fractionate the short-chain dextrins formed by the organism on the basis of their solubility in ethanol, after having first removed the glucose and maltose by fermentation and the ions on an exchange column. In the present work an examination has been made of the higher saccharides formed by the Monod strain of *Esch. coli* under conditions similar to those used by the American workers.

The products obtained from the incubation of *Esch. coli* with maltose were fractionated on a charcoal column (Whistler & Durso, 1950) into glucose, maltose and oligosaccharide fractions. The oligosaccharides were shown to contain three to eight glucose residues by semi-micro methods involving (i) periodate oxidation, (ii) alkaline iodine oxidation and (iii) a determination of the molecular weights of their acetates. Chromatographic examination of the products of β-amylolysis of the oligosaccharides demonstrated the reluctance of the enzyme to attack any residual trisaccharide moiety remaining after the normal mechanism of β-amylolysis had been completed.

Methylation of the total oligosaccharide fraction gave a product having OMe, 49·8%. A paper chromatographic examination of the hydrolysate of this methyl ether revealed only 2:3:6-trimethyl- and 2:3:4:6-tetramethyl-D-glucose. This evidence, together with the absence of any dimethyl glucose, leads us to infer that the oligosaccharides contain only 1:4-α-linkages and are devoid of branching.

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Non-diffusible 'Cores' Prepared from Various Deoxypentose Nucleic Acids. By S. Laland. (*Chemistry Department, University of Birmingham*)

It has been demonstrated both by Overend & Webb (1950*a*) and Chargaff & Zamenhof (1950) that the action of pancreatic deoxyribonuclease upon calf thymus deoxyribonucleic acid (DNA) leads to the formation of a non-diffusible residue or 'core'. This contains an increased ratio of purine to pyrimidine bases when compared with the parent DNA and is more resistant than the latter to further enzymic degradation. In a wide study of deoxypentose nucleic acids from various animal, plant and microbial materials under progress in this department, 'cores' have been prepared from highly polymerized preparations of DNA isolated from the following sources: calf thymus, soft herring roe, wheat germ, *Mycobacterium tuberculosis* (human strain) and *Myco. phlei*. The method of preparation was as follows: enzymic digestion of DNA with pancreatic deoxyribonuclease (McCarthy, 1946) for 16 hr. at 37° in a veronal buffer (pH 7) containing magnesium ions was followed by prolonged dialysis of the digestion mixture in a cellophan bag against distilled water (changed at noted intervals) at room temperature. The dialysis residues or 'cores' obtained comprised from 20 to 67% of the original DNA. The purine and pyrimidine composition of these 'cores' and the original DNA preparations were determined by a method of chromatographic analysis on paper and will be reported. The 'cores' obtained from DNA of calf thymus, herring roe and wheat germ exhibited an increase in the ratio of purine bases to pyrimidine bases when compared with the original DNA. This lends support to the view advanced by Overend & Webb (1950*b*) that the deoxyribonuclease preferentially hydrolyses pyrimidine nucleotides. The 'cores' obtained from the two microbial DNA preparations, however, showed a decreased ratio. This latter finding may originate in differences in the structure of these two microbial deoxypentose nucleic acids and the other nucleic acids examined.

In accordance with results obtained by Wyatt (1951), it has been demonstrated that 5-methyl-

cytosine is present in DNA from calf thymus, herring roe and wheat germ. Moreover, in those deoxypentose nucleic acids which contained this base, it was found that it persisted in the 'core', thereby supporting the view that this base is a true constituent of some deoxypentose nucleic acids.

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DEMONSTRATIONS

An Electronically Controlled Tensiometer for the Study of Changes in Surface Tension. By R. K. Bayliss and W. F. R. Pover (introduced by H. G. Sammons). (*Department of Pharmacology, University of Birmingham*)

A tensiometer has been arranged to measure the tension at the interface of oil and water during the lipolysis of the oil by pancreatic enzymes. The force required to pull the ring a small way above the level of the interface is measured by this device. Changes in this force correspond to changes in interfacial tension.

The force is measured as a current to a high degree of accuracy. This current is automatically adjusted by the apparatus to maintain the ring at a fixed distance above the position of the undisturbed interface. Graphs of current against time show the change in interfacial tension during the lipolysis.

Horizontal Paper Chromatography. By P. Meredith and H. G. Sammons. (*Department of Pharmacology, University of Birmingham*)

In the separation of water-soluble phospholipin hydrolysates using water miscible solvents, it was difficult to maintain an adequate homogeneous concentration of solvent in the vapour phase. This has been overcome by using a glass trough $12 \times 9{\cdot}5 \times 3$ in. in which is placed a glass frame carrying up to four strips of paper. The strips are of the usual dimensions 24×2 in. crossing the frame twice. Solvent covers the bottom of the trough into which the paper dips. At no time is the paper more than 1·5 in. above the surface of the solvent. The whole trough is covered with a sheet of glass sealed with silicone grease. The main advantages are: (*a*) adequate solvent concentration in the vapour phase, (*b*) economy of space.

Apparatus Used for Study of the Kinetics of Chemical Reactions *in vivo*. By H. G. Bray, W. V. Thorpe and K. White. (*Department of Physiology, The Medical School, University of Birmingham*)

An Apparatus for the Estimation of Water and Electrolyte Exchange in Rats. By D. F. Cole and S. J. Tranter. (*Department of Anatomy, The Medical School, University of Birmingham*)

Paper Chromatography of Keto Acids. By S. M. Altmann, E. M. Crook and S. P. Datta. (*Department of Biochemistry, University College, London*)

Cavallini, Frontali & Toschi (1949*a*,*b*,*c*) have reported the separation of keto acids by paper chromatography of their 2:4-dinitrophenylhydrazones using *n*-butanol as mobile phase. The spots were streaky, however, and the method failed for the phenyl keto acids. As the identification of the latter is of prime importance in the study of phenylketonuria, a flow-chromatographic method on alumina has been developed for their separation from urine (Datta, Harris & Rees, 1950). It was desirable to supplement this slower technique, however, and a detailed study of the conditions has made it possible to separate on paper the dinitrophenylhydrazone as compact spots and to obtain accurate and reproducible estimates of their R_F values.

(3) *Solvent.* *n*-Butanol with aqueous ethanol gave good results, but most satisfactory was tertiary amyl alcohol with aqueous ethanol. Under these conditions, the R_F values in Table 1 were found at 48 hr. after the atmosphere of the tank had been equilibrated with the stationary phase overnight. Shorter periods of equilibration may give rise to streakiness.

(4) *Mode of application of derivatives to paper.* 25 μg. of the dinitrophenylhydrazone in 100 μl. of buffer is a suitable quantity for application to the paper. The use of organic solvents, especially ethyl acetate, for this purpose often results in decarboxyla-

Table 1. *The paper chromatography of keto acids*

	R_F values					
	Tert-$C_5H_{11}OH$:EtOH:H_2O			*n*-C_4H_9OH:EtOH:H_2O		
Keto acid	50	10	40	50	10	40
Oxaloacetic acid	0·08			0·28		
α-Ketoglutaric acid	0·16			0·26		
Pyruvic acid	0·36			0·36		
Acetoacetic acid	0·50			0·43		
p-Hydroxyphenylpyruvic acid	0·70			Diffuse		
Phenylpyruvic acid	0·78			Diffuse		
o-Hydroxyphenylpyruvic acid	0·81			Diffuse		

The following were found to be critical.

(1) *Type of paper.* Whatman nos. 1, 4 and 54 were unsuitable. Only Whatman no. 2 gave good spots under the conditions to be described.

(2) *Pre-treatment of the paper.* Impregnation with alumina (Datta, Overell & Stack-Dunne, 1949) or washing with dilute acetic acid or ethylenediaminetetraacetic acid to remove heavy metals gave no improvement. Washing with buffer solutions gave good results provided the buffers were soluble in the mobile phase. The most successful buffer was glycine-NaOH, 0·1 M, pH 8·2–8·4.

tion (e.g. the dinitrophenylhydrazone of acetoacetic acid is converted almost completely to the dinitrophenylhydrazone of acetone). Application in phosphate buffer, 0·2 M, pH 7·2, prevents this, but even then the solutions do not keep for more than a day. Even with these precautions, the hydrazones of pyruvic acid and phenylpyruvic acid show second spots whose origin is at present unknown.

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Experimental Studies in the Sprue Syndrome. By A. C. Frazer, J. M. French and H. G. Sammons. (*Department of Pharmacology, The Medical School, University of Birmingham*)

FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal*:

The component acids of the perinephric and interscapular fats of a rabbit. By G. Clément and M. L. Meara

Studies in the biochemistry of micro-organisms. 84. The colouring matters of *Penicillium nalgiovensis* Laxa. Part 1. Nalgiovensin and nalgiolaxin. Isolation derivatives and partial structures. By H. Raistrick and J. Ziffer

The influence of a high-sucrose diet on the calcium and phosphorus percentage of the rat femur, and a comparison with its effect on the enamel and dentine of the rat incisor teeth. By R. L. Hartles

Studies on the metabolism of the Protozoa. 1. Metabolism of the parasitic flagellate, *Trypanosoma lewisi*. By J. F. Ryley

The dimensions of the particle of hyaluronic acid complex in synovial fluid. By A. G. Ogston and J. E. Stanier

A micro-method for the estimation of uronic acids. By A. G. Ogston and J. E. Stanier

The reduction of glutathione by plant tissues. By L. W. Mapson and D. R. Goddard

Studies on the enzymic oxidation of succinic acid containing deuterium in the methylene groups. By M. B. Thorn

Synthesis of milk fat from acetic acid ($CH_3\,{}^{14}COOH$) by the perfused, isolated bovine udder. By A. T. Cowie, W. G. Duncombe, S. J. Folley, T. H. French, R. F. Glascock, L. Massart, G. J. Peeters and G. Popják

The turnover number of cholinesterase. By W. K. Berry

The preparation and properties of β-glucuronidase. 2. Centrifugal fractionation of water homogenates and the nature of the sedimentable fraction. By P. G. Walker and G. A. Levvy

Degradative studies on the 'core' resulting from chymotryptic digestion of insulin. By Joan M. L. Stephen

The spectrophotometric determination of ascorbic acid in tissue extracts, particularly those of the walnut (*Juglans regia*). By C. Daglish

The occurrence of ascorbic acid in the walnut (*Juglans regia*). By C. Daglish

Non-protein nitrogenous constituents of rye-grass: ionophoretic fractionation and isolation of a 'bound amino-acid' fraction. By R. L. M. Synge

Paper chromatography of amines. By J. M. Bremner and R. H. Kenten

β-(3:4-dihydroxyphenyl)ethylamine (hydroxytyramine) in normal human urine. By U. S. von Euler, U. Hamberg and S. Hellner

The cytochrome system of adrenal medulla. By C. L. Tsou

Further observations on the *in vitro* stimulation by insulin of fat synthesis by lactating mammary gland slices. By Judith H. Balmain and S. J. Folley

The formation of β-ketoadipic acid by bacterial fission of aromatic rings. By B. A. Kilby

Comparison of haem *a*, the dichroic haem of heart muscle, and of porphyrin *a* with compounds of known structure. By R. Lemberg and J. E. Falk

An improved procedure for the determination of carbon in organic compounds in aqueous solution. By P. J. Heald

The oxidation in liver of L-tyrosine to acetoacetate through *p*-hydroxyphenylpyruvate and homogentisic acid. By W. E. Knox and M. LeMay-Knox

Some factors affecting anthocyanin synthesis in cress seedlings. By B. P. Eddy and L. W. Mapson

The nitration of benzoic and phenylacetic acids and phenylalanine: the chemical basis of the Mohler and Kapeller-Adler reactions. By C. T. Beer, F. Dickens and Dorrit Salmony

The action of sulphydryl inhibitors upon *iso*citric dehydrogenase with especial reference to the behaviour of some trivalent arsenicals. By W. D. Lotspeich and R. A. Peters

The biological action of substances related to thyroxine:

2. Further derivatives of 3:5-diiodo-4-hydroxybenzoic acid. By J. H. Wilkinson, Mary M. Sheahan and N. F. Maclagan

3. Substances derived from 3:5-diiodo-4-hydroxybenzaldehyde and related compounds. By J. H. Wilkinson, Mary M. Sheahan and N. F. Maclagan

Comparative studies of 'bile salts'. 3. The conversion of pythocholic acid to deoxycholic acid. By G. A. D. Haslewood

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **299th Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University of Sheffield, Sheffield* 10, *on Saturday,* 21 *July* 1951, *when the following papers were read:*

COMMUNICATIONS

The Metabolism of Azobenzene and *p*-Hydroxyazobenzene in the Rabbit. By H. G. Bray, R. C. Clowes and W. V. Thorpe. (*Department of Physiology, Medical School, University of Birmingham*)

The fate of the azo (—N=N—) group in the animal body is of interest because rupture of this linkage may give rise to compounds with conjugable centres so that the azo group could act as a 'potential centre for conjugation' (Bray, Ryman & Thorpe, 1948). Failing rupture of the azo group, conjugation with glucuronic acid or sulphuric acid could be achieved by introduction of a new hydroxyl group by hydroxylation. Previous workers have shown that the linkage can be broken, e.g. in azobenzene by the rat (Elson & Warren, 1944), and in *p*-dimethylaminoazobenzene by the rat (Stevenson, Dobriner & Rhoads, 1942; Miller, Miller & Baumann, 1945). Hydroxylation of the intact azobenzene molecule has not been observed.

In the present investigation an attempt has been made to assess the extent of the cleavage of the —N=N— linkage and degree of hydroxylation of azobenzene and some of its derivatives in the rabbit. Azobenzene is not completely absorbed, about 30 % of a dose of 0·5 g./kg. being found in the faeces. Approximately 60 % of the absorbed azobenzene was excreted as glucuronide and 20 % as sulphate (these values calculated on the basis of 1 mol. conjugating acid/mol. azobenzene) and 23 % unchanged. Corresponding values for *p*-hydroxyazobenzene, which was completely absorbed, were: unchanged, 3 %; glucuronide, 95 %; and sulphate, 27 %. The following metabolites have been identified in the urine after administration of azobenzene (figures in parentheses indicate percentage of dose isolated, other compounds were detected by paper chromatography): excreted free, *p*-hydroxyazobenzene (1·5), *o*-hydroxyazobenzene, benzidine (2), *p*-acetamidophenol, *o*-acetamidophenol, hydrazobenzene; excreted conjugated with glucuronic or sulphuric acids, *p*-hydroxyazobenzene (3), benzidine, *p*-aminophenol (5), *o*-aminophenol. The following were found after administration of *p*-hydroxyazobenzene: excreted free, *p*-acetamidophenol, *o*-acetamidophenol; and conjugated, *p*-aminophenol (57), *o*-aminophenol. Other derivatives of azobenzene are being examined.

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The Metabolism of 2:3:5:6-Tetrachloronitrobenzene and 2:3:4:5-Tetrachloronitrobenzene in the Rabbit. By H. G. Bray, Z. Hybs, H. J. Lake and W. V. Thorpe. (*Department of Physiology, Medical School, University of Birmingham*)

2:3:5:6-Tetrachloronitrobenzene (TCNB) is only absorbed to a small extent by the rabbit. With doses from 0·1 to 3·0 g., 60–80 % can be recovered unchanged from faeces; even a dose of 10 mg. is not completely absorbed. The TCNB which is absorbed is mainly excreted as glucuronide and mercapturic acid (12 and 7 % of dose respectively). Tetrachloroaminophenol (m.p. 186°) and the mercapturic acid (m.p. 207°) have been isolated from the urine. No toxic effects were observed.

The asymmetrical isomer of TCNB, 2:3:4:5-tetrachloronitrobenzene, caused anorexia in doses of 0·7 g. Absorption is greater than with the symmetrical isomer, only one-third of a dose being found in the faeces. A considerable part of the unabsorbed material is reduced in the gut to the amine which, unlike that of TCNB, readily diazotizes and couples. The absorbed compound is partly converted into glucuronide and ethereal sulphate (27 and 7 % of dose respectively). 2:3:4:5-Tetrachloroaniline was isolated from the urine (m.p. 118–120°). No mercapturic acid formation was detected.

Leuconostoc citrovorum Factor and the Synthesis of Serine by Micro-organisms. By JUNE LASCELLES, M. J. CROSS and D. D. WOODS. (*Department of Biochemistry, University of Oxford*)

It has been shown (Lascelles & Woods, 1950) that pteroylglutamate and pyridoxal are essential for the synthesis of serine from glycine and formate (in the presence of glucose) by washed cells of *Streptococcus faecalis* R deficient in these factors. Formylpteroylglutamate was 30% more active than pteroylglutamate. *Leuconostoc citrovorum* factor is replaced for growth of that organism by a synthetic tetrahydro derivative of formylpteroylglutamate ('leucovorin') (Brockmann, Roth, Broquist, Hultquist, Smith, Fahrenbach, Cosulich, Parker, Stokstad & Jukes, 1950), which was therefore tested in the above system. A sample of this material was kindly provided by Dr T. H. Jukes. It replaced pteroylglutamate, but was not more active when added simultaneously with the other components. However, cells first incubated in glucose-phosphate with leucovorin synthesize serine more actively than cells similarly treated with pteroylglutamate when tested in the serine synthesis system with or without pteroylglutamate.

When serine synthesis in the above systems is promoted by pteroylglutamate it is accompanied by formation of *L. citrovorum* factor. Such synthesis requires only formate, glucose and pteroylglutamate; the concentration of formate necessary is less than that required for serine synthesis.

It was also found (Lascelles & Woods, 1950) that the serine requirement for growth of *Leuconostoc mesenteroides* could be replaced by high concentrations of glycine provided that pyridoxal was present and the atmosphere was enriched with CO_2. It is now found that there is a quantitative relationship between concentration of glycine and partial pressure of CO_2, which suggests that, with growing cultures, CO_2 may act as precursor of the one-carbon unit condensed with glycine or a derivative. Leucovorin replaces the requirement for added CO_2; the low concentration necessary makes it impossible that the formyl group of this compound serves directly as source of the one-carbon unit.

Leucovorin also replaces *p*-aminobenzoic acid for growth of *L. mesenteroides* in either the presence or absence of serine. It overcomes sulphonamide inhibition of growth in a non-competititive manner. Neither pteroylglutamate nor its formyl derivative is active in these respects.

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Adaptation of _Bacillus subtilis_ to Fatty Acids. By H. LASER. (*Molteno Institute, University of Cambridge*)

Vaccenic acid

$$[CH_3.(CH_2)_5.CH{=}CH.(CH_2)_9.COOH],$$

which occurs in, and has been isolated from, a number of animal organs (Laser, 1949; Morton & Todd, 1950), has previously been shown to inhibit growth and respiration of *Bacillus subtilis* (Laser, 1950, 1951). The inhibition is transient at suitable concentrations of acid, respiration and growth ultimately attaining the same, or slightly higher, rates than those of untreated controls. Organisms which have recovered from the inhibition are resistant to further addition of acid. This phenomenon, which resembles acquired drug resistance by bacteria, has been further studied on cells treated with stearic, oleic, vaccenic and linoleic acid. The main results are as follows: (1) The acids tested inhibit growth and respiration qualitatively in a similar manner, but quantitatively in different degrees (stearic acid < oleic acid < vaccenic acid < linoleic acid). (2) At suitable concentrations recovery from the inhibition occurs with time, both in growing cells and in washed suspensions. (3) Recovered cells are resistant to considerably higher concentrations of acid than the initially inhibiting dose. (4) Treatment with any one acid produces resistance against any of the other acids. (5) A time lag ensues on treatment with a fatty acid before resistance occurs. (6) Low concentrations of acid, which do not inhibit growth or O_2 uptake, nevertheless produce resistance. (7) The tolerance of growing cells with subsequent development of resistance is slightly greater than that of washed suspensions. (8) The resistance is lost on subculturing the micro-organisms in absence of added fatty acid.

The evidence suggests that the resistance of treated cells is due to the development of an adaptive enzyme, which is formed subsequently to the

incorporation into the cell and metabolic degradation of the fatty acid. It therefore appears possible that the adaptive enzyme is produced by a mechanism resembling an immunological reaction, i.e. in response to the formation of a conjugated antigen, which arises from the intracellular combination of a preformed protein with a partial degradation product of the substrate.

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Effect of Carbon Monoxide on the Level of Energy-rich Phosphate in Surviving Muscle.
By R. A. Lawrie. (*Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge*)

The effect of carbon monoxide (CO) on the energy-rich phosphate ($\sim$P) levels of pre-rigor horse psoas muscle has been studied. For this purpose $\sim$P was taken to be the sum of the labile phosphorus of creatine phosphate and adenosinetriphosphate. The latter value was obtained by correcting '$P_{7\ min.}$' for triose- and hexose-diphosphates. Inorganic phosphate was estimated by the method of Allen (1940), after controlled hydrolysis of the various fractions. A series of gas mixtures, representing selected $CO:O_2$ ratios, was prepared, and to these were exposed, in sealed vessels at room temperature, thin layers (2–3 mm.) of minced horse psoas muscle from the first to the seventh hour post-mortem. The minces were then extracted by ice-cold trichloroacetic acid (TCA), the extracts being immediately neutralized. Control TCA extracts were made simultaneously from samples exposed to corresponding mixtures containing $N_2:O_2$. The $\sim$P content of these extracts was expressed as percentage total soluble phosphorus and the effect of CO represented as the difference between test and control values. In the dark, test samples at $CO:O_2$ ratios between 10:90 and 80:20 were found to have higher $\sim$P levels than those in corresponding $N_2:O_2$ controls, the difference being most marked when $CO:O_2$ was about 20:80. When the concentration of CO was greater than 0·8 atm. the $\sim$P level of the test extract dropped considerably below that of the corresponding control. (In 100% CO, however, the $\sim$P value was virtually the same as that in 100% N_2.) A similar but less marked drop occurred when the CO concentration was less than 0·1 atm. The stimulatory effect is no longer evident on illumination.

In the hope of accentuating this phenomenon during conditions when post-mortem glycolysis is greatly accelerated, an analogous series of analyses was performed on pairs of TCA extracts prepared from psoas muscle homogenates, 1 hr. post-mortem, through which had been passed standardized quantities of each $CO:O_2$ or $N_2:O_2$ mixture, for 2 min. before TCA addition. Once again the above effects of CO were apparent, being rather more marked than in the minces.

Glycogen and pH determinations suggest that the rise in $\sim$P is related to increased respiration. Partial inactivation of cytochrome oxidase is presumably responsible for the depression of $\sim$P levels at high CO tensions. The relation of these findings to the Pasteur effect, and to the hypothetical Pasteur enzyme, was briefly discussed.

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Vitamin B_{12} Activity for Chicks and different Micro-organisms of Gut Contents and Faeces.
By M. E. Coates, J. E. Ford, G. F. Harrison, S. K. Kon, J. W. G. Porter (*National Institute for Research in Dairying, University of Reading*), W. F. J. Cuthbertson and H. F. Pegler (*Glaxo Laboratories Ltd., Greenford, Middlesex*)

In our laboratories tests for vitamin B_{12} with *Escherichia coli* (*Bacterium coli*) (Bessell, Harrison & Lees, 1950) and *Lactobacillus leichmannii* ATCC, 4797 (Skeggs, Nepple, Valentik, Huff & Wright, 1950) agree between themselves for a variety of natural materials. Assays of aqueous extracts (prepared by steaming for 30 min. at pH 5 and filtration) of gut contents and faeces of calves and chicks receiving diets containing little or no vitamin B_{12} form a notable exception. Thus tests with fresh calf

faeces gave results of 0·36 μg. vitamin B_{12}/g. with *Esch. coli* and 0·07 μg. with *Lb. leichmannii*. The addition of cyanide (cf. Wijmenga, Veer & Lens, 1950) during extraction increased the results to 1·9 and 0·19, probably by facilitating extraction, since the potency of the aqueous extracts themselves so treated remains unchanged. Accordingly, cyanide treatment was used for all the extracts tested microbiologically. Tests with fresh chick droppings and chick caecal contents gave results with *Esch. coli* of 1·0 and 7·0, and with *Lb. leichmannii* of 0·27 and 1·65 μg. vitamin B_{12}/g., respectively.

A test with *Euglena gracilis* gave a value for dried rumen contents of 3·6, which agreed with the result of 3·8 with *Esch. coli*; the result with *Lb. leichmannii* was 1·1 μg. vitamin B_{12}/g.

Chick tests, which normally give in our experience values higher than the microbiological (Coates, Harrison & Kon, 1951), yielded a result of 0·2 for the dried rumen contents. A sample of dried calf faeces gave results of 10·6 and 1·9 with *Esch. coli* and *Lb. leichmannii*, and 0·4 μg. vitamin B_{12}/g. with chicks. These low values are not due to any depression of the growth of the chicks by the materials tested.

The activity measured by both *Esch. coli* and *Lb. leichmannii* is largely cell-bound in the original material but completely ultrafiltrable in the extracts, where it is destroyed by autoclaving at 15 lb. for 15 min. at pH 11·6. Satisfactory recoveries of added vitamin B_{12} were obtained from extracts before and after alkali treatment.

E. coli was grown in an extract of calf faeces. The harvested cells contained approximately the original ratio of activities for *Esch. coli* and *Lb. leichmannii*.

A method based on the *Esch. coli* cup-plate assay showed that the diffusion coefficients of vitamin B_{12} and the factor in faeces active for *Esch. coli* were approximately the same.

Work on the microbiological, biological and chemical aspects of the problem is continuing.

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The Effect of Antibiotics on the Growth of Chicks deprived of Vitamins of the B Complex.

By M. E. Coates, C. D. Dickinson, G. F. Harrison and S. K. Kon. (*National Institute for Research in Dairying, University of Reading*)

It was shown by Stokstad & Jukes (1950) and confirmed by us (Coates, Harrison, Kon, Mann & Rose, 1951) that antibiotics stimulate the growth of chicks on diets adequate in known vitamins.

To study the effect of the drugs in deficiencies of the B vitamins, groups of 12–16 day old Rhode Island Red × Light Sussex cockerel chicks received the deficient diets of Coates, Kon & Shepheard (1950) either alone or supplemented with penicillin (2·5 mg./100 g.) as the procain derivative. The experiments lasted 4 weeks, or less if high mortality intervened.

The mean final body weights (g.), with the numbers of surviving birds in parentheses, were at 4 weeks: complete diet 340 (15), with penicillin 370 (15); without biotin 311 (15), without biotin, with penicillin 348 (15); without nicotinic acid 225 (15), without nicotinic acid, with penicillin 200 (15); at 3 weeks: without folic acid 104 (13), without folic acid, with penicillin 164 (13); without pantothenic acid 76 (8), without pantothenic acid, with penicillin 84 (9); without riboflavin 70 (13), without riboflavin, with penicillin 73 (15); at 2 weeks: without pyridoxin 56 (8), without pyridoxin, with penicillin 67 (13). Birds receiving no aneurin, with and without penicillin, were all dead by the 12th day.

No vitamin B_{12} was added, since our normal birds usually carry reserves of this factor (Coates, Harrison & Kon, 1949), but further tests were done, in two stages, with its addition at the rate of 2 μg./100 g. diet. The results were, in the first stage, at 4 weeks: complete diet 328 (12), with penicillin 404 (11), with vitamin B_{12} 357 (12), with penicillin and vitamin B_{12} 381 (12); without biotin 254 (9), without biotin, with penicillin 286 (10), without biotin, with vitamin B_{12} 250 (11), without biotin, with penicillin and vitamin B_{12} 272 (11); without nicotinic acid 268 (11), without nicotinic acid, with penicillin 205 (11), without nicotinic acid, with vitamin B_{12} 199 (12), without nicotinic acid, with penicillin and vitamin B_{12} 216 (12); at 3 weeks: without folic acid 108 (3), without folic acid, with penicillin 143 (11), without folic acid, with vitamin B_{12} 102 (4), without folic acid, with penicillin and vitamin B_{12} 189 (11). In the second stage, at 3 weeks: complete diet 236 (16), with

penicillin 228 (16), with vitamin B_{12} 240 (15), with penicillin and vitamin B_{12} 243 (16); without pantothenic acid 70 (7), without pantothenic acid, with penicillin 68 (10), without pantothenic acid, with vitamin B_{12} 79 (8), without pantothenic acid with penicillin and vitamin B_{12} 82 (5); without riboflavin 59 (10), without riboflavin, with penicillin 71 (15), without riboflavin, with vitamin B_{12}, 68 (12), without riboflavin, with penicillin and vitamin B_{12} 71 (15); at 2 weeks: without pyridoxin 52 (3), without pyridoxin, with penicillin 64 (5), without pyridoxin, with vitamin B_{12} 53 (2), without pyridoxin, with penicillin and vitamin B_{12} 61 (4). Birds receiving no aneurin, with and without penicillin and vitamin B_{12}, were all dead by the 11th day.

The vitamins studied fell into three classes. The presence of the antibiotic had no effect on the degree of deficiency of aneurin, riboflavin, pyridoxin or pantothenic acid, lessened that of biotin or folic acid and increased that of nicotinic acid. Although there were indications that the diets were slightly deficient in vitamin B_{12}, the effects of penicillin were essentially the same when vitamin B_{12} was added. On two occasions growth on the supposedly complete diet was improved by addition of the antibiotic, but on the third occasion this improvement was not noted in a 3 week test.

The tendency to lesser mortality among the birds on the diets containing the antibiotic supports the theory that the drug acts by combating a minor infection, but the results with biotin, folic acid and nicotinic acid give strong evidence in favour of its altering the microbial population of the gut. That vitamin B_{12} also increases the severity of nicotinic acid deficiency suggests that it also, in this instance, may encourage the growth of organisms that use nicotinic acid at the expense of the host.

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The Formation of Fatty Acids by a Gram-negative Coccus. By S. R. Elsden, F. M. C. Gilchrist, D. Lewis and B. E. Volcani.* (*Department of Bacteriology, University of Sheffield, and the Division of Plant Nutrition, University of California, Berkeley*)

A strictly anaerobic Gram-negative coccus, isolated from the rumen of sheep, ferments lactate, glucose, fructose, sorbitol and mannitol with the production of volatile fatty acids and gas. The fermentation products from lactate were H_2 and CO_2, acetate, propionate, *n*-butyrate and *n*-valerate with traces of *n*-caproate (hexanoate). Glucose yielded H_2 and CO_2, acetate, *n*-butyrate and *n*-caproate with small amounts of propionate and *n*-valerate.

Washed suspensions of the organism suspended in phosphate buffer (pH 6·5) and under a gas phase of N_2 produced a gas other than CO_2, presumably H_2, from glucose, fructose, sorbitol, mannitol, lactate, pyruvate, formate, DL-alanine and DL-serine. Towards the end of the fermentation of pyruvate there were signs of a gas uptake; and when pyruvate was fermented under an atmosphere of H_2 this secondary uptake of gas was much increased, the pressure returning almost to its initial value by the end of the experiment. Addition of either acetate, propionate, or *n*-butyrate to the fermentation of pyruvate under H_2 increased the utilization of this gas. Analysis of the fermentation fluids for fatty acids by both paper chromatography (Brown, 1950; Hiscox & Berridge, 1950) and the quantitative liquid-gas partition chromatogram of James & Martin (1951) showed that the fermentation of pyruvate yielded acetate, *n*-butyrate and *n*-caproate with small amounts of propionate and *n*-valerate. Addition of acetate about doubled the yields of *n*-butyrate, *n*-valerate and *n*-caproate; addition of propionate gave a tenfold increase in the amount of *n*-valerate; and addition of *n*-butyrate increased the yield of caproate sixfold. There was at the same time a disappearance of the added fatty acid. These results suggest that a C_2 fragment from pyruvate combines with the added fatty acid and the resulting compound is then reduced by the hydrogenase system. These observations are in agreement with the work of Stadtman & Barker (1949) in which it was shown that extracts of a different organism, *Clostridium kluyveri*, converted a mixture of acetyl phosphate and acetate to butyrate and caproate in the presence of H_2.

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The Metabolism of Creatine by a Pseudomonad. By G. Appleyard (introduced by D. D. Woods).
Department of Biochemistry, University of Oxford)

Nimmo-Smith (1949) isolated an organism, provisionally identified as *Pseudomonas eisenbergii*, which can produce an adaptive system of enzymes enabling it to grow on creatine as sole source of carbon and nitrogen. Washed suspensions of organisms grown on a creatine medium were able to remove creatine; but they could not degrade any other tested guanidine derivative, except agmatine and arginine, which were attacked relatively slowly.

It is now found that washed cell suspensions rapidly oxidize creatine. The creatine removal and oxygen uptake continue at a uniform rate until the creatine has completely disappeared. At this stage the oxygen consumption falls, but never to the value without substrate. One molecule of urea is formed per molecule of creatine removed. The oxygen uptake and ammonia and carbon dioxide production (at the stage when creatine has disappeared) are about 55, 30 and 45 %, respectively, below the theoretical values for complete oxidation of the creatine, but no other product has been found. The occurrence of oxidative assimilation is indicated by the fact that these deficits are reduced about 50 % in the presence of azide.

Sarcosine is also oxidized by cell suspensions. At low concentrations the rate of oxygen uptake is only about 20 % of that with equimolar creatine. Raising the sarcosine concentration increases the rate, until it equals that with optimal concentrations of creatine.

Under anaerobic conditions, cell suspensions remove creatine with the formation of one mole of urea; chromatographic evidence suggests that the other product is sarcosine and estimations with an organism which oxidizes this substance indicate that one mole is formed. At the creatine concentration normally used, creatine is removed anaerobically at only about 20 % of the aerobic rate; by increasing the creatine concentration, the anaerobic rate of removal may be brought up to the optimal aerobic rate.

A soluble cell-free enzyme can be obtained by acetone-drying the cells. This enzyme hydrolyses creatine to one molecule of sarcosine (evidence as above) and one molecule of urea. Evidence has been obtained that the enzyme requires the presence of —SH groups for its activity.

It is suggested that the first stage in the aerobic breakdown of creatine by this organism is a hydrolysis to sarcosine and urea. The fact that, at low substrate concentrations, the rate of aerobic removal is considerably greater than the anaerobic rate suggests the possibility that, under aerobic conditions, creatine is actively concentrated within the cell.

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The Oxidation of Thymine by an Unidentified Bacterium. By R. D. Batt and D. D. Woods.
(*Department of Biochemistry, University of Oxford*)

Wang & Lampen (1951) have reported briefly on the oxidation of pyrimidines by a soil organism, and it may be useful to give an interim report on similar work which has been in progress here.

Two types of aerobic, rod-shaped bacteria (Gram-positive and non-sporulating) have been isolated by enrichment culture; they can utilize pyrimidines as sole source of carbon and nitrogen for growth. Cell suspensions of one organism (ST) oxidize pyrimidines even when grown on tryptic digest broth; with the other organism (SS), however, adaptive enzymes are involved.

Washed suspensions of SS derived from growth on either uracil or thymine attack both these pyrimidines but not cytosine. When grown on uracil the cells attack thymine (in phosphate buffer) at 30° with the initial accumulation of a compound which is subsequently (after a slight lag) further oxidized.

The point of inflexion in the O_2-uptake curve corresponds to an O_2 consumption of 0·5 mol. O_2/mol. thymine; no thymine is then present and only negligible amounts of NH_3, CO_2 and urea. The compound has an absorption peak at 268 mμ. and apparently a higher $\epsilon_{max.}$ value than thymine. It is unstable in acid conditions giving rise to material which does not absorb in the ultraviolet. Examination of paper chromatograms by the ultraviolet technique of Markham & Smith (1949) showed a very low R_F value with butanol-water, but a higher value (*c.* 0·3) with *iso*propanol-NH_3.

In the absence of phosphate, cell suspensions of SS may oxidize thymine in a different manner. In either water alone or in borate buffer the O_2 consumption curves are of a different type and there is less accumulation of the above absorbing compound; these differences are accompanied by changes in pH. Addition of phosphate buffer at various times during oxidation in water changes the O_2-uptake curve to the phosphate type provided thymine is still present.

Thiothymine (4-hydroxy-2-mercapto-5-methyl pyrimidine) is also oxidized in phosphate with an uptake of 0·5 mol. O_2 and with production of an ultraviolet absorbing compound. Thymine glycol (2:4:5:6-tetrahydroxy-5-methyl-5:6-dihydro pyrimidine) is not attacked. The evidence so far obtained is consistent with the view that the intermediate compound formed during thymine oxidation in phosphate buffer is uracil-5-carbinol (2:4-dihydroxy-5-hydroxymethyl pyrimidine) or a derivative.

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Further Studies on Porphobilinogen. By PHYLLIS E. BROCKMAN and C. H. GRAY. (*Department of Chemical Pathology, King's College Hospital Medical School, London, S.E.* 5)

The conversion of porphobilinogen to porphyrin and other pigments has been studied by following the changes in absorption spectra of purified porphobilinogen solutions heated at 100° at pH 5·2. Grieg, Askevold & Sveinsson (1950) have shown that maximum yields of porphyrin are obtained from porphobilinogen-containing urines on heating at this pH. After heating for 2 min. the solution became yellow in colour and showed two absorption maxima, a strong one at 477–480 mμ. and a much weaker one in the region of 350–370 mμ. On heating for a further 3 min. the absorption at these wavelengths increased, and the Ehrlich reaction was then negative. On further heating, the porphobilinogen solution became pink in colour and the absorption band at 477–480 mμ. diminished in intensity and a new maximum appeared at 408 mμ. The 408 mμ. band reached its maximum after 60 min. heating and then decreased. Throughout the whole period of heating there was a gradual increase in the general absorption. The original absorption band at 286–288 mμ. (Gray, 1951) remained unchanged except for a slight increase due to the increased general absorption, suggesting that this absorption band was due to some substance other than porphobilinogen. It now appears probable that this band was due in large part, if not entirely, to uric acid which had not been completely separated despite repeated chromatography on alumina.

The absorption maxima at 477–480 and 350–370 mμ. could be due to a dipyrrylmethene, while the 408 mμ. maximum is due to the formation of a porphyrin.

Lowry, Schmid, Hawkinson, Schwartz & Watson (1950) claimed that porphobilinogen does not give rise to porphyrin, and postulate a second precursor which can be separated from porphobilinogen. In the urines we have examined so far, there has been no evidence of the existence of this second precursor.

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The Chromatography of Steroid Benzoates. By R. V. Brooks, W. Klyne and E. Miller. (*Postgraduate Medical School, London, W.*12)

Brooks, Klyne & Miller (1951), in studies on the steroids of pregnant mares' urine, reported the separation of some isomers of pregnanediol by the chromatography of their benzoates on alumina. Turner & Voitle (1951) have also reported the separation of epimeric 20-hydroxysteroids as their benzoates. The chromatography of benzoates has not been widely used by previous workers in the steroid field (but see, for example, Goldberg & Wydler, 1943; Pearlman, 1944; Barton & Miller, 1950). We have now found that many pairs of related benzoates can be separated well by chromatography.

The following pairs of compounds have been studied using alumina (Savory & Moore Ltd.) of activity II–III (Brockmann & Schodder, 1941) and light petroleum (b.p. 60–80°)—benzene mixtures of gradually increasing benzene content. (*a*) 20-Epimers: the *allo*pregnane-3β:20-diols, the pregnane-3β:20-diols and the pregn-5-ene-3β:20-diols; in each case the two epimers separated well, the 20β-isomers being eluted first. (*b*) 3-Epimers: the androstan-3-ol-17-ones, androsterone and *iso*androsterone; good separation (androsterone eluted first). (*c*) 5-Epimers: androstan-3β-ol-17-one and aetiocholan-3β-ol-17-one; partial separation (androstanolone eluted first). (*d*) 17-Epimers: the androst-5-ene-3β:17-diols (as 3-acetate 17-benzoates); good separation (17α-isomer eluted first). (*e*) 5-*allo*- and Δ^5-compounds: androstan-3β-ol-17-one and androst-5-en-3β-ol-17-one; no separation.

The following benzoates have been prepared; m.p.'s are corrected; figures in square brackets are specific rotations in $CHCl_3$ at 20–25°. Monobenzoates: aetiocholan-3β-ol-17-one, m.p. 243°, [+79°]; pregna-5:16-dien-3β-ol-20-one, m.p. 171–172°, [−9°]. Dibenzoates: pregnane-3β:20α-diol, m.p. 168–169° (cf. Pearlman, 1944), [+23°]; pregnane-3β:20β-diol, gum.

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The Metabolism of Collagen from Skin, Bone and Liver in the Normal Rat. By J. C. Perrone* and H. G. B. Slack.† (*National Institute for Medical Research, Mill Hill, London, N.W.*7)

In previous communications (Perrone & Slack, 1951; Neuberger, Perrone & Slack, 1951) the metabolism of collagen from normal rat tendon was studied. The results indicated a very slow rate of turnover in adult rats. These experiments have been extended to include collagen obtained from tendon, skin, bone and liver in three groups of rats of initial weight ranges 50–70, 200–240 and 350–370 g. Each rat received glycine labelled with ^{14}C in the methylene group in amounts equivalent to 10 μc./100 g. body weight. Collagen was obtained free from other proteins: (1) in tendon by a modification of the method of Bowes & Kenton (1948); (2) in skin and liver by preliminary cleaning and mincing and then extraction with 20 % (w/v) urea followed by washing with distilled water; (3) in bone by grinding, decalcification with 6 % (v/v) HNO_3, extraction by 20 % (w/v) urea followed by treatment with trypsin and subsequent washing with distilled water. All collagen samples were converted to gelatin, filtered, and the glycine present was obtained after hydrolysis as crystalline 2:4-dinitrophenyl derivative using chromatography on buffered Celite columns.

Specific activities were measured in counts/min./2 sq. cm. disk of infinite thickness. In the old rats the activities in collagen from tendon and liver were very low. Skin and bone collagens were rather more active, but all were below 100 counts/min. The levels of activity were maintained over a period of 7 weeks. In the young rats collagen glycine from all four sources gave counts of 816–944/min. at 1 week. There was some loss of activity during the first

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3 weeks, but very little further loss in activity at 8 weeks (after correcting for increases in body weight). The observed activities in all collagen samples in the young rats at 1 week were at least 10 times those from the old rats. In the rats of 200–230 g. the activities of the collagen samples were more variable, liver and bone collagen being 4–5 times as active as collagen from tendon and skin at 1 week. The interpretation of the activities found in the 200–230 g. rats is difficult since the exact ages of these rats were not known. The results indicate some metabolic activity in collagen from skin, bone, tendon and liver during the first 3 weeks in young rats, very little activity in old rats at any time, and variable metabolic activity in the 'young adult' group.

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Effect of 2:4-Dinitrophenol on Glycolysis in Mammary Gland and in Brain Tissue. By C. Terner. (*National Institute for Research in Dairying, University of Reading*)

Lactating rat mammary gland slices, incubated with glucose under anaerobic conditions, produced lactic acid at slow rates ($Q^{N_2}_{CO_2}$, +2 to +3 μl./mg. dry wt./hr.). The rate of glycolysis was accelerated by nicotinamide (0·01–0·02 M) and further increased and stabilized by pyruvate (0·001–0·05 M). In the presence of glucose, pyruvate and nicotinamide, high rates of anaerobic glycolysis were observed which remained almost linear for up to 3 hr. ($Q^{N_2}_{CO_2}$, about +10). Enzymic inactivation of cozymase, which is prevented by nicotinamide (Mann & Quastel, 1941), may explain the rapid decline of anaerobic glycolysis, previously observed by Folley & French (1949).

Under aerobic conditions only small amounts of lactic acid were formed by rat mammary tissue metabolizing glucose ($Q_{glucose}$ about −4, $Q_{lactic\ acid}$ less than +1. Q_X = μl./mg. dry wt./hr.; X determined chemically). Addition of 5×10^{-5} M-2:4-dinitrophenol (DNP) resulted in 30–40 % inhibition of $Q_{glucose}$, but at the same time an increased accumulation of lactic acid occurred in amounts equivalent to 50 % of the glucose metabolized. Mammary gland slices differ in this respect from brain cortex slices, in which $Q_{glucose}$ and $Q_{lactic\ acid}$ were increased concomitantly by even higher concentrations of DNP (2×10^{-4} M). In the presence of L-glutamate (0·01 M), the addition of both cozymase (5×10^{-5} to $1{\cdot}25 \times 10^{-4}$ M) and DNP (2×10^{-4} M) increased the rate of aerobic glucose breakdown to its anaerobic level ($Q_{glucose}$ −8·0), thus completely inhibiting the Pasteur effect. Although, in brain tissue, the increased aerobic lactic acid formation might be due to the stimulation of the rate of glucose breakdown by DNP, this cannot be the case in mammary tissue in which $Q_{lactic\ acid}$ is increased while $Q_{glucose}$ is inhibited. DNP does not inhibit $Q_{pyruvate}$ and does not increase the formation of lactic acid from pyruvate in mammary tissue (see Terner, 1951*a*).

It has been suggested (Terner, 1951*b*) that DNP causes increased operation of the oxidation-reduction step of glycolysis by inhibition of the aerobic reoxidation of reduced cozymase at the triosephosphate level. This mechanism would explain the stimulation of lactic acid formation by DNP in the absence of an increased breakdown of glucose. On this assumption the present findings would also appear to be in favour of an explanation of the Pasteur effect, at least in brain tissue, in terms of a competition between oxygen and pyruvate for the hydrogen of reduced cozymase (Ball, 1939).

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Paper Chromatography of Urinary Acids and Phenols. By R. J. Boscott (introduced by H. G. Sammons). (*Department of Anatomy, University of Birmingham*)

Phenols and acidic fractions obtained from urines by hydrolysis with hydrochloric acid or β-glucuronidase are separated into four main fractions by partition between organic solvents and aqueous phases. The extracts are separated into components using two-dimensional paper chromatography (Boscott, 1951). Aqueous alkali of high salt concentration is used as the first solvent, no organic solvent phase being present. *n*-Butyl or *n*-amyl alcohol is employed for the second solvent. Whatman no. 542 paper and the ascending technique is used.

Components are detected by (i) their fluorescence in ultraviolet light before and after spotting the paper with droplets of 90% (v/v) H_2SO_4, and (ii) their reaction with a diazonium reagent.

Results obtained by applying these procedures to human and animal urines were described.

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Urinary Components in Rats and Human Subjects with Fibrosis of the Liver. By K. Aterman and R. J. Boscott (introduced by H. G. Sammons). (*Department of Anatomy, University of Birmingham*)

In a series of experiments designed to study the role of steroid compounds in liver disease, the chromatographic technique of Boscott (1951) was applied to the separation of certain urinary components. Different chromatographic patterns were obtained in groups of rats with normal and fibrotic livers before and after the administration of steroid hormones and other compounds.

The possibility of applying the new technique to human material was investigated in a small preliminary series. In the three cases of fibrosis of the liver examined, a component was found which was not present in the urine of a few patients with other diseases and in normal urine. Small amounts of this compound were also found in one case of steatorrhoea, and in one case of toxaemia of pregnancy.

In urines from members of a family with hepatolenticular degeneration (Wilson's disease) detectable amounts of the same compound have been found even in early cases, although in one of these patients no significant liver damage was revealed by routine investigations.

Further investigations are in progress.

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Colorimetric Determination of Arylsulphatase. By D. Robinson, J. N. Smith and R. T. Williams. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W.* 2)

Arylsulphatase (phenolsulphatase) can be conveniently determined using, as substrate, potassium 2-hydroxy-4-nitrophenylsulphate (0·0025 M) made from 4-nitrophenol by the Elbs persulphate oxidation (Smith, 1951). This ethereal sulphate on hydrolysis yields 4-nitrocatechol, which on making alkaline gives a red colour ($\lambda_{max.}$ 510 mμ., $\epsilon_{max.}$ 11,300). Tissue extracts were incubated at 37° for an hour with the substrate in acetate buffer at pH 6. Protein was removed, if necessary, with phosphotungstic or trichloroacetic acid, and NaOH was then added. The red colour obtained was measured in the Spekker absorptiometer whereby 4–30 μg. of 4-nitrocatechol were readily estimated.

Defining a nitrocatechol unit of arylsulphatase activity as that amount of enzyme which will liberate 1 μg. of 4-nitrocatechol in 1 hr. at 37° at pH 6 in acetate buffer, the relative activities of 1 g. of various materials were found to be as follows: takadiastase, 850; rat tissues, spleen, 300–400; lung, 100–200; liver, 25–70; heart, 70–80; kidney, 60–110; intestine, 30–50. We have also examined the 'saliva' of locusts (*Locusta migratoria migratorioides*) and found it to contain 800–1000 units/ml.

of arylsulphatase activity. None was found in human saliva.

The action of takadiastase on two ethereal sulphates yielding nitroquinol on hydrolysis has also been studied. Nitroquinol in alkali gives a purple but unstable colour ($\lambda_{max.}$ 540 mμ., $\epsilon_{max.}$ 3850). 4-Hydroxy-2-nitrophenylsulphate is readily hydrolysed by takadiastase, but its isomer 4-hydroxy-3-nitrophenylsulphate is not.

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Sucrose Breakdown by Enzyme Preparations from Moulds. By F. J. Bealing and J. S. D. Bacon. (*Department of Biochemistry, University of Sheffield*)

The invertase of *Aspergillus oryzae* has been regarded as a 'glucosaccharase', because it is inhibited by free glucose and fails to attack raffinose (cf. Neuberg & Mandl, 1950; Gottschalk, 1950). An investigation of the action of various mould enzyme preparations on sucrose has shown that this view is probably incorrect.

In a typical experiment a dialysed extract of *A. oryzae*, obtained by autolysis under toluene, was incubated with 7·5% sucrose in 0·01M-acetate buffer (pH 5·0), and the reaction mixture analysed by quantitative paper partition chromatography (Bacon & Edelman, 1951). After 3 hr. at least 80% of the sucrose had disappeared, but only 7·5% of the fructose and 42·5% of the glucose expected from simple hydrolysis was present in the free state; the remainder appeared in the form of non-reducing substances with R_F values less than that of sucrose. These compounds were hydrolysed after incubation for several days, giving only fructose and glucose; they were not identical with those produced by yeast invertase (Blanchard & Albon, 1950; Bacon & Edelman, 1950).

The rate of formation of free fructose was not influenced by variations in the sucrose concentration, nor by the addition of glucose. The rate of incorporation of fructose in oligosaccharides other than sucrose was trebled when the initial sucrose concentration was raised from 8 to 40%; in the presence of glucose it was substantially reduced.

The above results may be explained by assuming that the enzyme preparation transfers fructose residues from sucrose to any carbohydrate molecules present in the reaction mixture, and also to water; and that the products of these transfer reactions, with the exception of free fructose, then act as substrates for further transfer reactions, the reaction ending when all fructose residues have been transferred to water. This hypothesis is supported by the observation that when sucrose is incubated with mould enzyme preparations in the presence of various alcohols, fructose-containing substances are formed having R_F values greater than that of free fructose; a similar observation has been made with yeast invertase. That a single enzyme is responsible for both hydrolytic and transferring activities is suggested by the fact that preparations from different species, from mycelia of different ages, and from crushed spores (Hughes, 1951), give rise to quantitatively similar mixtures of free sugars and oligosaccharides during their action on sucrose solutions of the same concentration.

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The Metabolism of ^{14}C-labelled Bicarbonate in the Cat. By H. L. Kornberg, R. E. Davies and D. R. Wood. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry; and Department of Pharmacology and Therapeutics, University of Sheffield*)

In the course of studies on the metabolism of labelled urea in the cat (Davies & Kornberg, 1950) it was necessary to obtain information on the rate of excretion of $^{14}CO_2$, and on the rate of incorporation of ^{14}C into urea, after injection of ^{14}C-labelled bicarbonate. Much work on the former topic has been published in recent years, but this was usually carried out on mice (Skipper, White & Bryan, 1949;

Skipper, Nolan & Simpson, 1950) or rats (Armstrong & Schubert, 1949; Armstrong & Zbarsky, 1949; Gould, Sinex, Rosenberg, Solomon & Hastings, 1949; Greenberg & Winnick, 1949; Schubert & Armstrong, 1949), in which the $NaH^{14}CO_3$ was injected intraperitoneally. However, this method of administration of the isotope, and the differences in body size between mice and rats, and cats, made it impossible to apply the earlier results to our studies.

Cats were anaesthetized with ether and chloralose (75 mg./kg. body wt.) and connected through a valve and cannula inserted in the trachea to a special respiration circuit by means of which the cat freely inspired CO_2-free air, the expired CO_2 being continuously collected in 15% NaOH for definite lengths of time. The ureters were tied and $NaH^{14}CO_3$ ($5–7 \times 10^5$ counts/min.) injected intravenously. The rate of expiration of $^{14}CO_2$ is initially very rapid, and can be expressed as an exponential equation with three components, the curves obtained in our experiments corresponding to the function:

$$P = 28{\cdot}4e^{-0{\cdot}243t} + 41{\cdot}5e^{-0{\cdot}0234t} + 30{\cdot}2e^{-0{\cdot}00119t},$$

where P is the percentage of injected ^{14}C left in the cat at time t. In the first 30 min. 50% of the injected isotope is expired, by which time the blood bicarbonate had lost all but 5%. The total excretion over 5 hr. was 80–82%. The rate of incorporation of ^{14}C into urea follows closely the rate of expiration of $^{14}CO_2$. After 5 hr. the urea carbon contained 3·1% of the injected ^{14}C; over 50% of this was incorporated in the first 40 min. Our data show that within the experimental error (±2%) all the urea carbon is derived from CO_2 in equilibrium with blood bicarbonate. This confirms the results obtained *in vitro* by Grisolia & Cohen (1948) and *in vivo* by Mackenzie & du Vigneaud (1948).

The results show that there are several 'CO_2 cycles' operating, since the injected $NaH^{14}CO_3$ equilibrated rapidly with the total blood bicarbonate and at varying rates with other carbon pools including the bicarbonate in the tissues. After 5 hr. 10–12% of the ^{14}C injected had been incorporated into the tissues in forms which exchange relatively slowly with the bicarbonate of the blood. Most of this was present in bone.

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Sources of Error in the Assay of β-Glucuronidase in Animal Tissues. By P. G. Walker and G. A. Levvy. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

Considerable attention has been paid to comparative measurements of the glucuronidase activity of different tissues, or of a single tissue in different normal and pathological states. Most methods of assay make use of the supernatant obtained by adding acetic acid or citrate or acetate buffer to the water homogenate, and incubating for varying periods before centrifuging. The introduction of phenolphthalein glucuronide as substrate (Talalay, Fishman & Huggins, 1946) has made possible the use of the untreated water homogenate.

In work already reported in part (Kerr, Levvy & Walker, 1950; Kerr & Levvy, 1951), it was found that after homogenizing mouse liver in water some of the glucuronidase activity was associated with subcellular particles. Acidification led to agglutination and rapid sedimentation of the particles. In dilute preparations, the overall homogenate activity was equal to the sum of the soluble and insoluble fractions. The insoluble enzyme gradually went into solution on incubation in acetate buffer, but not in citrate buffer, nor in preparations acidified with acetic acid. Various other measures have been found to bring the insoluble enzyme into solution, for example, addition of the surface-active agent Triton X-100 or mechanical disintegration in the Waring blender.

From the study of homogenates in isotonic media it has been concluded that by far the greater part of the glucuronidase activity in mouse liver is originally associated with subcellular particles. Such homogenates did not show their full glucuroni-

dase activity on direct assay, and required preliminary activation for reliable measurement. All comparable measurements have, however, been in agreement in confirming the observation of Levvy, Kerr & Campbell (1948) that in the mouse the glucuronidase activity is higher in infant liver and in liver regenerating after partial hepatectomy than in the normal adult tissue.

Preliminary results in a similar investigation of rat liver suggest that this tissue contains both soluble and insoluble glucuronidase, but otherwise behaves very differently from mouse liver in the enzyme assay. Water homogenates of rat liver did not initially display their full activity (as realized by adding Triton X-100) in acetate buffer, and the discrepancy was very much greater in infants than in adults. Full activity was displayed by adult, but not by infant liver, after preliminary incubation in the buffer for 2 hr. In citrate buffer, true values were always obtained for adult liver, and with infant liver the discrepancies were less marked than in acetate. On our present data, any opinion as to the relationship between the glucuronidase activity of rat liver and its state of proliferation would be premature.

This work has obvious bearing on the comparative measurements of other enzymes.

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An Amylolytic Degradation of Rabbit-liver Glycogen. By D. J. Bell and D. J. Manners. (*School of Biochemistry, University of Cambridge*)

A large batch of glycogen (unit chain length by both periodate and methylation methods, 12; $[\alpha]_D$ in water +198°, and mol. wt. by sedimentation-diffusion (Bell, Gutfreund, Cecil & Ogston, 1947), approx. $4{\cdot}5 \times 10^6$) was subjected to stepwise degradation by the β- and α-amylases of barley. At pH 4·6, β-amylase (free from α) gave a high mol. wt. dextrin (L.D. 1) (74 %) and maltose (26 %). L.D. 1 gave a yellow-brown colour with iodine, its solution in water was opalescent and had $[\alpha]_D + 194°$. In N-NaOH $[\alpha]_D$ was +159° while the parent glycogen had $[\alpha]_D + 169°$. The unit chain length by periodate was 9. Mol. wt. by light-scattering (through the co-operation of Dr B. S. Harrap of the Department of Colloid Science) was $3{\cdot}9 \times 10^6$ compared with the value found for the parent glycogen, namely $5{\cdot}9 \times 10^6$. The 'reducing powers' (as maltose) of L.D. 1 and the glycogen were respectively, (*a*) by copper method, nil and nil, (*b*) by hypoiodite, 3·4 % and 2·7 %. Total N (Kjeldahl) was 0·05 % for the glycogen and 0·12 % for L.D. 1.

L.D. 1 treated with α-amylase (free from β-) at pH 5·4 gave a low mol. wt. dextrin, L.D. 2 (76 %) and 24 % of reducing sugars (maltose and a little glucose). L.D. 2 gave no colour with iodine; the aqueous solution was clear and $[\alpha]_D$ was +186°. Reducing power (as maltose) was (*a*) copper method, 12·3 %, (*b*) hypoiodite 14·0 %, 'degree of polymerization' (calc. from Cu reduction) was approx. 17 and from this an approximate mol. wt. of 2800 can be deduced. Total N (Kjeldahl) was 0·22 %.

L.D. 1 treated simultaneously with both enzymes at pH 5·4 gave 74 % conversion to a heterogeneous mixed dextrin (L.D. 3) along with maltose and some glucose. L.D. 3 gave no colour with iodine, gave a clear solution in water and had $[\alpha]_D + 179°$. Reducing power as maltose was (*a*) by copper method, 18·0 %, (*b*) by hypoiodite, 19·3 %. 'Degree of polymerization' (calc. from Cu reduction) was approx. 12 and from this an approximate mol. wt. of 2000 could be deduced. Total N (Kjeldahl) was 0·22 %.

In all digests the reaction was stopped when a constant copper reduction had been attained. This was measured by a slight modification of Somogyi's (1937) method. The dextrins were separated by ethanolic precipitation.

L.D. 3 was separated by dialysis into two fractions differing markedly in $[\alpha]_D$ and 'degree of polymerization', namely, approx. 6 and approx. 16, respectively. L.D. 3 was shown to undergo further degradation by salivary amylase.

Our results on β-amylolysis of a 12-radical glycogen are of interest when compared with the findings of Halsall, Hirst, Hough & Jones (1949) in the case of an 18-radical specimen. Both residual dextrins (i.e. L.D. 1) must have chain lengths of approximately nine to ten radicals and the glycogens therefore differ presumably with respect to the external chains. Halsall *et al.* used wheat and crystalline sweet-potato β-amylases. On the other hand, Meyer & Fuld (1941) found 50 % degradation

of an 11-radical glycogen from mussels; it would seem that this glycogen may differ from rabbit-liver polysaccharide with respect to the location of the point of branching.

The loss of an average of three glucose radicals per unit chain during β-amylolysis, together with the selective fission of certain interior chains by the α-enzyme, would be in accord with the view put forward by Meyer & Fuld (1941) that the glycogen molecule is not only highly branched but that the branches are irregular.

We wish to acknowledge valuable advice and help given by Dr C. S. Hanes, F.R.S., and Mr E. T. Whitmore in connexion with the enzymic work in the initial stages.

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The Amino Groups of Conalbumin, Ovomucoid and Avidin. By H. Fraenkel-Conrat (Rockefeller Foundation Fellow) and R. R. Porter (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

There occur in egg white four singular proteins of markedly specific biological activities. It appeared of interest to apply to these the techniques of Sanger (1945) and Porter & Sanger (1948) for the characterization and determination of amino groups. The proteins investigated were conalbumin, ovomucoid and avidin, lysozyme having been studied elsewhere (Green & Schroeder, 1951).

Conalbumin presented no unusual problems. It appeared to contain a single terminal amino group per mole, which was identified as alanine (1·0, average of four experiments, range 0·9–1·1). Since a greater number of peptide chains had been postulated on an analytical basis (Lewis, Snell, Hirschmann & Fraenkel-Conrat, 1950), unsuccessful attempts were made to reveal more end groups after various types of denaturation (found 1·2/mole, three experiments). The amount of ϵ-dinitrophenyl-lysine found was in good agreement with the microbiological assays for this amino acid (Lewis *et al.* 1950) (62 and 60 residues/mole).

The DNP-ovomucoid was soluble in water and aqueous ethanol, so that it had to be isolated by dialysis. The breakdown of dinitrophenyl-alanine and ϵ-dinitrophenyl-lysine when boiled for 16 hr. with 20 % HCl in the presence of ovomucoid was 40 and 30 %, respectively, higher than losses previously reported with other proteins (Porter & Sanger, 1948). This is presumably due to the high carbohydrate content of ovomucoid. When these corrections for losses are applied, one terminal alanine per mole was found (0·96, six analyses ranging from 0·8–1·2); the corrected lysine value is somewhat higher than the lysine found microbiologically (13·7 and 12 residues/mole).

With various avidin preparations, both nucleoprotein and nucleic acid free, rather erratic results were obtained. It appears, however, that all the lysine ϵ amino groups (26) react, and that there are three N-terminal alanine residues per mole.

Both with ovomucoid and with avidin, there was often a small amount of another slow-moving yellow component resembling glycine detectable on silica gel columns developed with chloroform, amounting always to less than half an equivalent per mole. It appears more likely that it represents an artifact, possibly characteristic of glycoproteins, rather than a genuine end group of a contaminating protein.

No evidence was found for reaction of fluoronitrobenzene with components of the nucleic acid of avidin, or with pure thymonucleic acid.

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The Absorption of Inorganic Phosphate from the Rumen of the Sheep. By R. Scarisbrick and T. K. Ewer. (*Department of Animal Pathology, University of Cambridge*)

The saliva of ruminants is voluminous and very rich in inorganic phosphate; this causes a large movement of phosphorus from the blood into the rumen, where the concentration is kept high. The present experiments were undertaken with sheep to see whether inorganic phosphate is absorbed from the rumen. The ruminal vein and the carotid artery of sheep were exposed under nembutal or chloral anaesthesia and the inorganic phosphate content of the blood in these vessels compared.

An injection of inorganic phosphate containing ^{32}P was made into the rumen of the first sheep: within 10 min. the isotope appeared in the blood in the ruminal vein and to a less extent in the blood in the carotid artery and was also present in the saliva. Subsequent samples of blood and saliva contained less isotope.

Phosphorus (2·8 g., as a mixture of sodium dihydrogen phosphate and disodium hydrogen phosphate at pH 6·4) was injected into the rumen of the second sheep. Five minutes after the injection the inorganic phosphate content of the blood in the ruminal vein had risen from 6·3 to 24 mg. P/100 ml. and in the carotid artery from 5·8 to 17 mg. P/100 ml.

These results indicated that absorption of inorganic phosphate from the rumen is possible and further determinations were carried out to assess the extent of such absorption when the contents of the rumen are not disturbed. Experiments on four more sheep showed that under these circumstances the amount of absorption was very variable, varying considerably in the same animal from time to time. The mean of all determinations of the arterio-venous difference of inorganic phosphate was only 0·24 mg. P/100 ml. The extent of the variability is shown by the high value of the standard deviation of the a.v. differences, 0·93 mg. P/100 ml.; on several occasions inorganic phosphate appeared to pass from the blood to the rumen. The net absorption of inorganic phosphate from the rumen over a long period of time, therefore, seems to be at most only small in amount, but at any particular instant there may be a substantial movement of inorganic phosphate into or out of the blood traversing the ruminal wall.

Acetate Metabolism of *Aerobacter aerogenes*. By S. Dagley, G. A. Morrison (*Department of Biochemistry, University of Leeds*) and E. A. Dawes (*Department of Biochemistry, University of Glasgow*)

Aerobacter aerogenes was trained to grow on aerated synthetic media utilizing ammonia as nitrogen source with acetate, succinate, fumarate or malate as sole sources of carbon, and rates of production of pyruvate, as determined by the method of Friedemann & Haugen (1943), were measured for aerated washed suspensions of the trained cells. Considerable training was necessary before acetate was utilized readily for growth: training to metabolize other substrates (e.g. glucose, malate, fumarate, succinate) did not confer adaptation to acetate (Baskett & Hinshelwood, 1950); and production of pyruvate by cells trained to utilize other substrates was feeble when aerated in acetate-phosphate buffer. When trained to grow on acetate, however, cells were not only adapted to succinate, fumarate and malate, but pyruvate was produced readily from acetate as well as from the dicarboxylic acids.

From our results it appears that the pathway by which acetate-grown cells produce the pyruvate for growth requirements may be the 'dicarboxylic acid cycle' suggested by Barron, Ardao & Hearon (1950) in their studies with *Corynebacterium creatinovorans* and supported by recent work with *Escherichia coli* using radioactive carbon (Ajl & Kamen, 1951).

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Baskett, A. C. & Hinshelwood, C. N. (1950). *Proc. roy. Soc.* B, **136**, 520.

Barron, E. S. G., Ardao, M. I. & Hearon, M. (1950). *Arch. Biochem.* **29**, 130.

Friedemann, T. E. & Haugen, G. E. (1943). *J. biol. Chem.* **147**, 415.

FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal:*

The precise determination of haemoglobin in blood by abridged photoelectric spectrophotometry. By J. W. Nicholas

The activation of a proteolytic enzyme in blood by animal tissue. By T. Astrup

Electrical stimulation *in vitro* of the metabolism of glucose by mammalian cerebral cortex. By H. McIlwain, G. Anguiano and J. D. Cheshire

Adenosinetriphosphatase activity of brain. By Marion B. R. Gore

Actions of electrical stimulation and of 2:4-dinitrophenol on the phosphates in sections of mammalian brain *in vitro*. By H. McIlwain and Marion B. R. Gore

The action of peroxidase systems on ferrocyanide, molybdate, tungstate and vanadate. By R. H. Kenten and P. J. G. Mann

The assimilation of amino-acids by bacteria. 13. The effect of certain amino-acids on the accumulation of free glutamic acid by *Staphylococcus aureus*: extracellular peptide formation. By E. F. Gale and Marguerite B. van Halteren

Studies in vitamin A:

15. Preparation of neovitamin A esters and neoretinene$_1$. By P. D. Dalvi and R. A. Morton
16. Spectroscopic properties of all-*trans*-vitamin A and vitamin A acetate. Analysis of liver oils. By H. R. Cama, F. D. Collins and R. A. Morton

Appendix—Correction procedure. By H. R. Cama, F. D. Collins and R. A. Morton

Separation and estimation of saturated C_2–C_7 fatty acids by paper partition chromatography. By R. L. Reid and M. Lederer

The relative rates of oxidation of lipovitellin and of its lipid constituent. By C. H. Lea and J. C. Hawke

Enzymes concerned in the synthesis of glycogen from glucose in the brown adipose tissue. By N. H. Creasey and C. H. Gray

Direct oxidation of glucose-6-phosphate, 6-phosphogluconate and pentose-5-phosphates by enzymes of animal origin. By F. Dickens and G. E. Glock

The digestibility of carotene by the cow and the goat as affected by thyroxine and thiouracil. By R. Chanda, Helen M. Clapham, Mary L. McNaught and E. C. Owen

The effect of thyroxine and thiouracil on the composition of milk. 1. The partition of phosphorus in cow milk in relation to phosphatase and other constituents. By R. Chanda and E. C. Owen

Non-peptide linkages in gramicidin. By A. T. James and R. L. M. Synge

The role of gastric urease in acid secretion. By R. E. Davies and H. L. Kornberg

The specificity of cobra-venom cholinesterase. By L. A. Mounter

Observations on the formation and structure of bacterial cellulose. By R. Kaushal, T. K. Walker and D. G. Drummond

A means of metabolic investigation of small portions of the central nervous system in an active state. By H. McIlwain

Further investigations on fish tropomyosin and fish nucleotropomyosin. By G. Hamoir

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